

How Much Would Rothschild Cost?

THE argument about the organization of the research councils in Britain seems at last to have come out into the open, and Sir Frederick Bawden's contribution to it (see page 7) is a foretaste of the kinds of disagreements that will be made public before the government eventually comes to a decision. So far, however, such arguments as there have been have tended to take at its face value Lord Rothschild's assertion that the problem of deciding how much effort should be spent on civilian research and development should for the time being be left on one side. It is of course true that it has in the past been difficult for governments anywhere to be sure whether they have found a wise balance between their instincts to spend as little as possible and the crying need to tackle all kinds of problems that would contribute to public welfare. In the past few years, there has been a tendency for governments to measure their performance by the proportion of the national income spent on research and development, but this is an unreliable yardstick. In the last resort, there is no escape from the much more difficult task of trying to work out ways of deciding whether money spent on particular research programmes is likely to be money well spent. Lord Rothschild's recipe for running the research councils does at least have the merit of providing means of evaluating particular proposals for research.

How would this empirical system work out in practice? Although the ministries are not entirely innocent of procedures for placing contracts with organizations outside the government, which is how the Rothschild recipe would have them deal with the research councils, their experience is concentrated in fields where something well defined must be procured. The Ministry of Defence is, naturally enough, the most conspicuous customer in the government, the Department of the Environment has inherited some expertise in the employment of contractors on the construction of buildings and other ministries are being encouraged to make more serious use of outside contracts as a means of learning more about the needs and the behaviour of the citizens with whom they have to deal. In all these situations, however, government departments are well placed to know in advance precisely what they wish to procure from outside. The novel difficulty in Lord Rothschild's recipe is that the kinds of research that ministries might wish to sponsor are necessarily less easily defined. The fact is that, as things are, most ministries are entirely ill-prepared to ask questions of the research councils with sufficient precision.

One immediate result is that anything like a full-blooded implementation of Lord Rothschild's recipe would probably create a situation in which the cost of work undertaken by the research councils would be increased. Consideration of any tangible example of the kind of project that might be let on contract to the councils will show how that would come about. The work done by the Natural Environment Research Council

on mineral resources in Britain is one convenient illustration. At present, the research council is spending £1.15 million on this work, all but £100,000 of it in its own research institutes. Lord Rothschild's argument is that the Department of Trade and Industry is formally responsible for the sponsorship of the mining industry, which seems to imply that it should be the organization which in future would put up a million pounds or more each year to keep mineral exploration going. It is hard to see how the Department of Trade and Industry will actually formulate its requirement of the research council for the continuation of work of this kind. At the same time, it is hard to think that the research council will be able to respond without increasing its charge to the ministry for the work it carries out.

Evidently it would be insufficient for the Department of Trade and Industry to say, "Carry out a programme of reconnaissance so as to assess the potential of mineral deposits". Any potential contractor would immediately ask for information about the scale on which the work was to be undertaken. Would it be necessary to survey the whole of Britain for deposits of sand and gravel within a year, or could the work be spread out over five years? Which minerals should in any case come near the top of the list of priorities? Is the customer more worried about potential shortages of sand and gravel than he is anxious to help find deposits of petroleum in the North Sea or deposits of precious or semi-precious metals in the old rocks of the Cambrian Shield? Once these questions have been asked, the Department of Trade and Industry will be compelled to decide why, in any case, it wants to know more about sand and gravel. Mere curiosity is not a sufficient justification for spending money. In any case, the practical need is not for an inventory of all the resources of gravel which might at some time be exploited. Rather, the objective should be an assurance of some kind that identified resources are sufficient to ensure that the construction industry will not grind to a halt uncomfortably soon.

Even the present government, with its doctrine that private industry should stand on its own feet, will acknowledge that such an investigation is legitimately a public responsibility. But how soon is uncomfortably soon? Is it sensible to include identified deposits in the forward inventory without also saying something of the cost at which they might be exploited? And, for the proper investigation even of such a practical problem, is it sensible to start work without an understanding of how gravel deposits have been formed geologically? If the dialogue between the customer and the contractor were in any way as creative as it should be, would not the outcome be a decision that the research contract should include funds for an economic investigation of the gravel industry and for a considerable amount of basic research? To be sure, much of the information required for these investigations may exist already, but it may not at present



be suitably organized. And after the work has been carried out, it would be necessary to keep in being, by means of a continuing research grant, some organization for keeping the inventory up to date.

The first thing to say is that the outcome could be valuable. Everybody would benefit from tangible practical studies of this kind. (To say this is not to overlook the competence with which the Institute of Geological Sciences is at present able to answer particular questions on mineral resources which may be put to it.) Because the product of the research would have to be some kind of document, and because this would have to be delivered at some specified time, the cost of the work would be greater than under present arrangements, which assume that useful bodies of knowledge like this will arise from comprehensive programmes of research undertaken by the Institute of Geological Sciences. In negotiating the price, the research council responsible would be well advised to cater for the possibility that the task would turn out to be more difficult than it first appeared, and would also presumably include an overhead charge to cover part of the cost of such general services as geological mapping which the IGS must in any case continue. In short, whatever the objections to the Rothschild recipe which there may be—the strongest of which centre on the capacity of the Civil Service to ask sensibly pointed questions of the research councils—one unexpected benefit could be that spending on research by the research councils would be substantially increased. Is the government prepared for that?

In case the government's courage should begin to drain away, it would be best if it could be encouraged to reflect on the wider issue—the question of how much it would be wise to spend on basic research of the kind now handled by the research councils. The total cost at present is £125 million a year, but there is no reason to think that they would be unable to spend twice as much and, in the process, to double the value of their contribution to the public good. To the extent that the Rothschild recipe—and the government's hasty endorsement of the customer-contractor doctrine—represents discontent with the quality (in the sense of pointedness) with what they do at present, productivity (whatever that may mean) should even be increased. The result is that full-blooded application of the Rothschild recipe should be taken as a commitment to a substantial upward revision of the budgets of the research councils, taken as a whole.

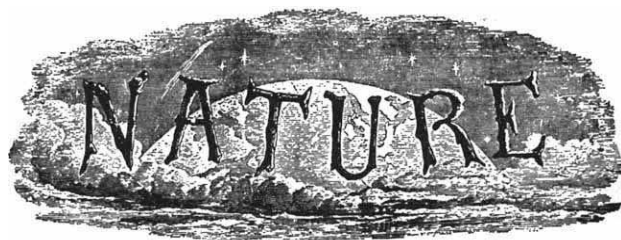
It is not difficult to see where the extra funds would go. It is inevitable that under present arrangements, the distribution of research council funds should be organized so as to spread what money that there may be among the money-spenders considered to be worthwhile. These arguments explain why the research council establishments are often less well equipped than would be comparable institutions dedicated to more explicit tasks. If the performance of the councils is in future to be determined by their performance on the projects which are specified by the contracts let to them, they will find themselves spending more on equipment and on outside services. The chances are that any economies which may be possible on manpower will be swamped by the necessarily greater cost of producing answers to well-chosen questions.

All this implies that the government cannot hope to push the research councils in the direction which the Rothschild reports points out without helping to support not merely the particular projects it may eventually

specify but the basic research without which sensible answers cannot be produced. It would in any case be a great mistake at this stage to dismantle the machinery for carrying out basic research which the research councils have been able to create. This, after all, is what most of the critics of Rothschild have been saying for the past few days, although, it must be acknowledged, those who have been attempting to argue, in the columns of the daily newspapers, that pure research whose pattern is determined by academic science is ineluctably beneficial have done as much to contribute to the case for change as any report from Lord Rothschild could.

But may it not be that Britain cannot afford such a large amount of basic research? That is a quite proper question. One of the weaknesses of the government's present position, for which Lord Rothschild is not to blame, is that the analysis which has been carried out of the place of basic research in British science policy has been innocent not merely of the needs of defence and of industry but of the opportunities for making better use of existing facilities within a European framework. Nobody would expect these deficiencies to be made good overnight, for the problems are teasing. But is that not another reason for asking that the first approach to Lord Rothschild's recipe should be a deliberate experiment in some field of research where the government departments might be expected to be able to ask sensible questions quite soon? That would give everybody time to consider the wider questions still unformulated.

100 Years Ago



THE UNITED STATES DEPARTMENT OF AGRICULTURE*

THE absence of a Department of Agriculture from the complicated scheme of British Government offices leads us to inquire whether it is possible for such a Department in the United States to publish annually eleven or twelve hundred pages of matter useful to the agricultural community, and whether those publications have any considerable circulation in the country.

The question of circulation is abundantly answered by a resolution of the House of Representatives passed on July 14, 1870 (the Senate concurring), which enacted, "That there be printed of the Annual Report of the Commissioner of Agriculture for 1869 *two hundred and twenty-five thousand extra copies*, one hundred and eighty thousand of which shall be for the use of the House, twenty thousand for the use of the Senate, and twenty-five thousand for distribution by the Commissioner of Agriculture." These figures are so startling in their magnitude that they seem to prove too much, until we recollect that the United States of America extend over an area proportionately enormous, including every gradation of climate, from the sub-tropical to the sub-arctic, and every variety of culture, from the cotton and rice of the south to the corn and roots of the north.

From Nature, 5, 197, January 11, 1872

OLD WORLD

ENVIRONMENT

Decay of Strontium-90

THE amount of radioactivity in the environment continues to decrease as it has done every year since 1963 according to three separate reports published in the past few weeks.

Radioactive Fallout in Air and Rain (HMSO, £0.40), a report by R. S. Cambray, E. M. R. Fisher, W. L. Brooks and D. H. Peirson of the Health Physics and Medical Division at the Atomic Energy Research Establishment at Harwell, shows that there has been little change in the deposition of the radionuclides caesium-137 and strontium-90 on the surface of the Earth since 1966. The Harwell team estimates that 0.21 megacuries of strontium-90 were deposited in all during 1970—some 20 per cent less than in 1969. This rate of deposition is, however, one tenth of that in the peak year of 1963. The cumulative deposition of ^{90}Sr since 1963 has increased from 6.90 megacuries to 12.37 megacuries, a total that has been substantially constant since 1966. This apparent inconsistency arises because the injection of new radioactive debris into the stratosphere from Chinese and French nuclear explosions—of which there have been three since 1970—has balanced deposition from it and the deposition of radioactive nuclei on the Earth's surface has made up for the decay of radionuclides already deposited. The Harwell team estimates that the Chinese explosion of October 1970 contributed 60 per cent of the long-lived activity in the air over Britain in the summer of 1971.

A complementary report to the Harwell document has been produced by N. T. Mitchell of the Fisheries Radiobiological Laboratory at Lowestoft on *Radioactivity in Surface and Coastal Waters of the British Isles, 1970*. The laboratory from which this report originates monitors the radioactive waste discharged from industrial and other sites. The report shows that all sites under surveillance discharged only a small proportion of their maximum allowed quota. Discharges from Winfrith, which is principally engaged on the development of new reactor systems, totalled only 4 per cent of its annual quota of 30,000 curies, whereas the nuclear power station at Trawsfynydd emitted 60 curies of tritium during 1970, well below the set limit of 2,000 curies. The atomic energy establishments at Harwell and Aldermaston did discharge a greater percentage of their allowed limits.

Of particular interest in the report are the estimates given of public radiation exposure from liquid radioactive waste during 1970. All the Central

Electricity Generating Board's establishments emitted less than 0.1 per cent of the dose limit set by the International Commission on Radiological Protection. The United Kingdom Atomic Energy Authority establishments also had a good record but the emissions from Windscale, in certain cases, were more of a hazard than the emissions from other sites. The estimated dose, for example, to a person who ate 160 g of laverbread every day during 1970 amounted to 5 per cent of the recommended dose limit—this estimate is based on the fact that a great deal of the seaweed used to manufacture laverbread comes from the Cumberland coast. Mitchell also estimates that the solitary person who supervises the salmon fishing operation in an estuary near Windscale was exposed to a dose of 12 per cent of the allowed maximum. This results from an accumulation of radioactive nuclei in the silt of the estuary and the fisherman is irradiated chiefly by gamma rays.

One of the most perplexing results in the report is the way in which over the past four years an increasing amount of ^{110m}Ag has been found in oysters near Bradwell power station in Essex. During 1970, the silver content was for the first time greater than that of ^{65}Zn which has decreased in recent years. The presence of the silver contaminant was first observed four years ago but according to Dr Mitchell the level has remained steady since the summer of 1970. In spite of this increase and the greater toxicity of silver, there has not been a significant increase in the human radiation dose from eating oysters. From a survey of the consumption of oysters it is concluded that an individual is only subjected to 0.1 per cent of the maximum permissible dose from this source.

The third report, on the *Assay of Strontium-90 in Human Bone in the United Kingdom* (HMSO, £0.185) for 1969, is produced by the Medical Research Council. The decrease of radioactive strontium in the atmosphere

since 1963 is reflected in the decreased amount found in the bones of babies and young children. The report shows, however, that there are regional variations and that there is considerably more radioactive strontium found in the bones of children in Glasgow than in children from elsewhere as a result of the greater rainfall in the district. The statistics show that in the 5–19 year old group, the mean ^{90}Sr value in 1969 was 1.8 picocurie g^{-1} calcium whereas the corresponding value for Glasgow was 3.9 picocurie g^{-1} calcium. Both these values are less than they were in 1968 and the downward trend is seen in all age groups.

EDUCATION

Counting the Cost

THE total public expenditure in England and Wales on education in 1969/70 was £1,979 million compared with £760 million in 1959/60. Local education authorities spent £1,705 million, of which 46 per cent was accounted for by teachers' salaries. And 79 per cent of the spending by the central government (£274 million) was channelled to universities through the University Grants Committee (*Statistics of Education, 5*, HMSO, 1971; £1.37).

During the 1960s the annual increase of expenditure on schools and further adult education increased at a fairly uniform rate of about 8.5 per cent and 12.5 per cent a year. The growth of spending on the training of teachers and on the universities, however, decreased during the decade; average annual increases were 24.3 and 22.0 per cent in the first five years, and 16.6 and 8.3 per cent respectively between 1965 and 1970. The total number of students undergoing teacher training in colleges of education in 1969–70 was 108,000 (4.5 per cent more than in the previous year) but the number of day students increased by 13 per cent to 41,200, indicating a continuing trend for greater numbers of mature students to enter the teaching profession.

Apart from awards for teacher training, local authorities spent an average of £325 a head on fees and maintenance for 251,500 students holding full value awards for courses in universities and at further education establishments. Although the proportion of 18 and 19-year-olds taking up awards from public funds for higher or further education continues to increase—it was 23.1 per cent in 1970 compared with 12.9 per cent in 1963—there is a considerable disparity between different parts of the country. In the London borough of Newham, for example, 25 per thousand of the age group took up local authority awards at universities during 1970 whereas in Solihull the corresponding figure was 185.

Knighthoods for Scientists

In the New Year Honours List, Sir Peter Medawar was made a Companion of Honour for his services to medical research. Professor Colin Buchanan, Imperial College, University of London, Professor Fred Hoyle, University of Cambridge, and Professor George Porter, Royal Institution, were made Knights Bachelors. Professor Alan Hodgkin, President of the Royal Society, was made a Knight Commander of the British Empire.

NEW WORLD

Dissent Muted at AAAS Jamboree

by our Washington Correspondent

Philadelphia, January

"SOME of us set the scene to make it unimportant" declared Senator Hubert H. Humphrey soon after he was narrowly missed by a tomato during his address to the annual meeting of the American Association for the Advancement of Science. Humphrey's complaint was that the antics of radical protesters at the meeting were stealing the headlines while what was being said by the speakers went largely ignored. But, as the meeting dragged on during the week between Christmas and the New Year, it became increasingly evident that few headlines would be grabbed by the radical dissenters, for their protests were more muted than at either of the two previous AAAS annual meetings and their presence was much less flamboyantly advertised.

Some of the radicals claim that the decline in their disruptive tactics is a conscious development — "everybody now knows that there are insurgents in science and there is now more of a need for educative activities" said one leading spokesman—while their opponents claim that the radical movement itself is on the wane. Others suggest that the AAAS has managed to forestall much of the more outspoken criticism which surfaced at the meeting in Boston in 1969 and in Chicago last year by arranging more political discussions and allowing the radicals to have their say. And yet another important factor was the escalation of the air war in Indochina, which provided a focus for dissent only tenuously linked with the AAAS meeting and which diverted the radicals' attention from the programmed sessions.

But at the start of the meeting, it seemed that the pattern of the two previous AAAS meeting would be repeated. The radical scientists, operating from a set of rooms provided by the AAAS, had picked a number of sessions on which to focus their activities. A session on health care in Philadelphia was the target of leaflets and a series of interruptions and questions which were for the most part well prepared and well articulated, and a session on technology and the humanization of work was the focus of rather less well prepared verbal criticism. But the chief target of the day's activities was Senator Hubert Humphrey, who was scheduled to address a symposium concerned with "value and knowledge requirements for peace".

Before the meeting started, the plat-

form on which Humphrey was due to speak was decked out with placards proclaiming him a war criminal, a murderer and a "pimp for US imperialism", and with the red clenched fist posters of the radical "Science for the People" movement. The stage was also occupied by a few protesters and by a swarm of reporters. The protesters agreed to leave the stage provided that the posters were allowed to remain, a condition which was accepted by the AAAS organizers ("you can keep your childish posters for all we care" said Athelstan Spilhaus, AAAS retiring president who marched on the stage) and Humphrey entered to give his address. He was greeted with some heckling, a few paper aeroplanes made from Vietcong flags, and with an otherwise neutral audience.

But soon after he started speaking, a large tomato hit the podium with a resounding thud and two protesters were promptly arrested and unceremoniously hustled out of the room by plainclothes policemen in the audience. That was the chief interruption of the session, and Humphrey was for the most part allowed to defend his past policies on the Vietnam war, inform the audience of his record of support for arms control legislation and join the protesters in denouncing the increased bombing of North Vietnam. The only incident which seemed to ruffle him was when he was asked to submit to a "citizen's arrest" for his complicity in "war crimes perpetrated against the people of Vietnam". He declined.

The upshot of that particular activity was that Humphrey was well pleased with the national television coverage that he received, and the protesters managed to get him to sign a statement to the effect that he will campaign for a definite date to end the war and that he will oppose support for the Thieu regime. But Dr Daniel P. Moynihan, a vice-president of the AAAS and a former adviser to President Nixon, was not pleased with the affair. The following day he issued a statement apologising to Humphrey for the treatment he received, and announced that he would not give his scheduled vice-presidential address. "In circumstances where Hubert Humphrey is not allowed to speak freely", he declared, "I choose not to speak at all". Moynihan called a press conference to explain his position and checked out of the hotel saying that he would save his address until next year. He had been mentioned the previous night in a meeting of radicals as a possible target for action, for had he not also worked at the White House?

The other main "happening" or-

ganized by the radicals at the meeting came on the final day, when William P. Bundy, Assistant Secretary of State for Far Eastern Affairs from 1964-69 was confronted by anti-war protesters denouncing his past record as an influential policy maker during the escalation of the war. Bundy was allowed to make his opening statement (on the theme "Conflict situations: Vietnam—Knowledge Gap") with a few interruptions, after which Leslie Gelb, of the Brookings Institution was scheduled to speak. The protesters, however, demanded that Bundy's statement should immediately be discussed and browbeat the Chairman, Morton H. Halperin, also of the Brookings Institution, into taking a vote on whether the meeting should immediately proceed to a discussion of Bundy's statement for an hour, followed by Gelb's address, or whether it should proceed as planned. The meeting overwhelmingly decided to embark on the discussion, postponing Gelb.

Bundy was subjected to some emotionally loaded and sometimes articulately expressed statements and questions for an hour. He stood up to the questioning well, and although he managed to convince few of his opponents, denounced the escalation of the bombing. But when the agreed questioning time was up, Halperin took back the microphone and handed it to Gelb amidst considerable heckling. Gelb found it impossible to proceed, and Halperin adjourned the meeting which ended in total confusion.

At a press conference immediately after the fiasco, Bundy said that he would have been willing to carry on ("the British would have considered that a tea party" he said). But Jeremy J. Stone, executive director of the Federation of American Scientists—a liberal and intellectually heavyweight lobby of scientists concerned chiefly with arms control—immediately weighed in with a statement condemning the radicals for their tactics and the AAAS for allowing the interruptions to take place. The AAAS, he said, should have taken down the posters at the meeting at which Humphrey spoke, ask disrupters to leave meetings and not permit them to return, and meetings should be stopped whenever heckling and disruption takes place.

Stone's attack on the radicals' tactics and Halperin's handling of the meeting (he is also a director of the Federation of American Scientists) neatly emphasize the differences between the radical protesters who campaign under the slogan "science for the people" and the

liberal FAS. What sets the two groups apart is essentially the fact that the FAS is prepared to work for liberal change within the present political system, while the chief thrust of the movement is that the system should be changed. The radicals will argue that the conduct of science cannot be separated from the political and economic system in which it is practised (see box) and that the system should therefore bear the brunt of their analysis and criticism. In short, they preach subversion and socialist revolution—a factor which sets them apart from the bulk of the AAAS membership.

But the objectives of the Science For People movement are scarcely evident from their tactics at the AAAS meetings. They are consistently regarded as disruptive rather than constructive, and their tactics at the meeting addressed by Humphrey served chiefly to raise the hackles of even those participants who were also opposed to the Vietnam war. Although Herbert Fox, of the Northeastern University, and a prominent member of SESPA (Scientists and Engineers for Social and Political Action) said at a press conference “we are not interested in alienating the people at this meeting”, that is often the chief product of their actions. To some extent, however, SESPA can claim credit for achieving a limited objective—making the AAAS into a rather more political body.

This year's meeting at Philadelphia contained many more political and social issues, ranging from a session on the new activism in science, to discussions of the contribution of science for peace and to sessions dealing with urban problems in Philadelphia. Spilhaus, who condemned the “childish graffiti” of the posters at the session addressed by Humphrey, admitted that “by some extremists we are pushed into the political arena”. But he quickly added that if there were no dissent and no forthright discussion at the AAAS meetings “it would mean that we are not relevant”. This shift in the content of some of the symposia is seen by many observers as a significant factor in the lessening of disruptive activity at the meeting this year.

Dr Barry Commoner, of Washington University and a board member of the AAAS, suggests, for example, that the association's policy of opening its doors to free discussion has had a positive effect on curbing disturbances, and Fox suggested that “the AAAS has found a way to discuss the same things we want to discuss”.

But another important reason for the diminished activities of radical scientists at Philadelphia was the escalation of bombing in North Vietnam, which started on the first day of the meeting and provided a target for the radical scientists' frustrations. All the main

disruptions at the meetings were channelled towards a symposium on Value and Knowledge Requirements for Peace, and one day's activities was taken up by a march through the centre of Philadelphia to protest against the war. Frustration with the war was also shared by many of the less radical participants at the meeting, and the dissenters found themselves with considerable common ground among other members of the AAAS. (At one evening meeting, for example, a Vietnam Veteran was given leave to address the meeting to ask for contributions to the bail fund for his colleagues arrested in the Statue of Liberty. He was greeted with joyous applause and generous donations.)

The dissenters are, however, considerably out of tune with the AAAS as a body, and vigorously attack the way the organization is run and the way that the meetings are organized. A handout published by Science for the People at the conference, for example, states “The AAAS structures its sessions so that the all knowing luminaries of science can illuminate a passive audience with the latest technological

solutions [to social problems]. But the role of the scientist should be to question the basic premises of the AAAS, to analyse the social context of scientific advance, and to further the political change necessary to resolve social problems. For the AAAS, success means more and continued privileges for scientists, it means that they continue to be well rewarded and secure priestly servants of corporate America”.

It is debatable, however, whether much of the “passive audience” will have been illuminated by the meeting of the AAAS. There were few important scientific advances announced at the meeting—other vehicles for scientific communication are much more effective—and most of the other sessions charted familiar grounds. The overriding impression was that it had all been said before, and that the meeting serves chiefly as a social occasion to fill the dull days after Christmas. The most commonly heard complaint was that the meeting is really just a huge spectacle. As one professor's wife remarked during an evening session “the whole thing is a circus—a tweedy circus”.

A Radical View

RADICAL dissenters at the AAAS annual meetings are frequently criticized for not stating their objectives and their beliefs, but for concentrating instead on confrontations with the scientific establishment. The following short extract is taken from an article describing the roots and the broad objectives of the Science for the People Movement. It was written by Bill Zimmerman, formerly Assistant Professor of Social Sciences at the University of Chicago, Len Radinsky and Mel Rothenberg, Associate Professor of Anatomy and Professor of Mathematics respectively at Chicago, and Bart Myers, Associate Professor of Psychology at Brooklyn College. The authors claim that the article was rejected by *Science* in spite of five referees' reports urging publication, and it was published by Science for the People and sold at the conference.

“In this society, at this time, it is not possible to escape the political implications of scientific work. The American ruling class has long had a commitment to science, not merely limited to short range practical applications, but based on the belief that science was good for the long-term welfare of American capitalism, and that

what was good for American capitalism was good for humanity. This outlook was shared by the trustees of universities, the official leaders of US science, the administrators of government and private funding agencies. Further, they see this viewpoint as representing a mature social responsibility, morally superior to the ‘pure search for truth’ attitudes of some of the scientists. But they tolerate that ideology since it furthers their own aims and does not challenge their uses of science.

“We find the alternatives of ‘science for science's sake’ and ‘science for progress and capitalism’ equally unacceptable. We can no longer identify the cause of humanity with that of US capitalism. We don't have two governments, one which beneficently funds research and another which represses and kills in the ghetto, in Latin America and in Indochina. Nor do we have two corporate structures, manipulating for profit on the one hand while desiring social equality and justice on the other. Rather there is a single government-corporate axis which supports research with the intention of acquiring powerful tools, of both the hard and soft-ware varieties, for the pursuit of exploitive and imperial goals.”

Conference Jottings

AMONG the results presented and the happenings which took place at the AAAS meeting last week are the following:

- Dr George O. Abell of the University of California at Los Angeles told a symposium that the universe may be twice as old as generally believed. He bases this suggestion on a study of eight galaxy clusters in which he measured the distance from Earth of several hundred individual galaxies. Using the "big bang theory", Abell estimated that some of the galaxies have travelled between 1.5 and 2 times as far as previously believed, extending the age of the universe between 15,000 and 20,000 million years.

- A symposium on the biological basis of destructive behaviour was told of studies on monkeys which suggest that aggression is not a learned behavioural trait, but on the contrary, it is the ability to control aggression which must be learned. Dr Allyn C. Deets of the University of Pittsburg and Dr Harry Harlow of the University of Wisconsin described experiments in which monkeys were raised in isolation during their early development. The monkeys were later unable to control aggressive behaviour directed at others and even at themselves. Dr Louis S. B. Leakey of the National Museum, Nairobi, disagreed with the suggestion, however. He pointed out that archaeological records show that aggression in man did not appear until about 40,000 years ago—after more than two million years of human evolution—and suggested that aggression started when man learned how to make fire, a discovery which radically altered the life style of early man.

- One of the targets of radical scientists was an article by Harvard psychologist Richard Herrnstein which was published recently in *Atlantic*. Herrnstein's thesis is that the economic stratification of Western society is inherited since the stratification into rich and poor is a product of inherited intelligence. Since Herrnstein was not present at the AAAS to defend his theory, radical scientists held discussion meetings to present their own analyses of his article and distributed numerous leaflets explaining why they disagreed with him. Part of the public education was a guerilla theatre, containing two songs, one of which, to the tune of "Ain't she Sweet", had the chorus:

"Ain't they sweet,
They're the ruling class elite,
and it's all determined quite
genetically,
Ain't they sweet."

- A panel considering the implications of the rising demand for energy sug-

gested the need for reducing the demand for power to conserve energy resources and to preserve the environment. The leading advocate for such a course of action was Dr Barry Commoner, of Washington University, St. Louis. Drawing on a study being conducted by the Scientists' Institute for Public Information, Commoner asserted that the use of electricity has been doubling every fourteen years, while the efficiency with which it is being used has been steadily declining, the chief reason being a switch to power-intensive processes for producing consumer products and the substitution of plastics for wood, aluminium for building materials, detergents for soap and so on. Commoner accepted the inevitable conclusion that his suggestion would reduce the productivity of labour, but argued that such a consequence may be the lesser evil. Scientists affiliated to the energy industry naturally took a different viewpoint, arguing that more energy is needed to reduce pollution and recycle wastes. The discussion followed close to the paths charted during recent discussions of Dr Commoner's book *The Closing Circle*.

- As the meeting dragged on, a noticeable phenomenon was the proliferation of lapel badges among the participants. Apart from the red clenched fist and the hand holding a laboratory flask, depicting affiliation to the Science for the People movement, participants wore badges proclaiming "I am aware", and "Save the Environment". One participant was even seen with a badge bearing the words "Sorry I missed your Paper".

BOMB DAMAGE

Unsung Destruction

by our Washington Correspondent

WHILE radical scientists were noisily venting their frustrations about the Vietnam war on Senator Hubert Humphrey at the AAAS meeting last week, a more coherent and perhaps more effective statement was being made by two biologists in a parallel session of the conference. Arthur H. Westing of Windham College, Vermont, and E. W. Pfeiffer of the University of Montana described in a session aptly entitled "The Social Consequences of Unsuitable Technologies", the environmental damage suffered by Indochina from craters and shrapnel. Both had visited Vietnam with the AAAS herbicide commission which reported to the annual meeting in 1970 but, as Westing later remarked in an interview, damage from bomb craters has been a sadly neglected area of potential ecological study.

Westing estimates that the 23,000 million pounds of munitions expended in Indochina between 1965 and 1970 have made about 23 million craters,

with an average width of 30 feet and a depth of 15 feet each. Nineteen million such craters are in South Vietnam alone. Based on the few figures released by the Department of Defense and on observations made during a visit to Vietnam in August 1971 sponsored by the Scientists' Institute for Public Information, these estimates suggest a scale of destruction that will have a lasting impact on the ecology of South-East Asia.

One environmental consequence is that the flying metal fragments from bombs and shells are hurled over an area much greater than that of the actual crater. The chief effect is damage to surrounding trees, which quickly succumb to fungal infections, and when badly damaged by fragments, are unusable for lumber. As for rubber trees, Westing says that the rot which enters through shrapnel wounds weakens the stems so that they simply break off in high winds. The damage from bombing and shelling thereby adds greatly to the environmental damage to Indochina from herbicides and bulldozers.

By far the greatest ecological bomb damage comes from the craters themselves. Westing estimates that each crater displaces on average some 200 cubic yards of soil, scattering the infertile subsoil over a large area. Persistent and worthless bamboo and other weeds often colonize the areas around craters in forested regions, making subsequent agriculture more difficult. Another effect is that the craters often will not fill by natural processes, and subsequent reclamation of the land must therefore be accomplished by laboriously filling them in by hand. (Westing described craters that were four years old with less than three feet of soil washed into the bottom.)

Many of the craters are filled with water during most of the year, providing breeding grounds for mosquitoes and presenting an additional threat to public health and increasing the difficulties in reclaiming the land for agriculture. A few of the craters which remain filled the whole year round may, however, be used for fish farms, which could help to offset some of the loss resulting from disruption of the intricate irrigation systems.

Perhaps the chief threat to the environment comes, however, from the fact that farmland that has been badly bombed or shelled is not recultivated because of the danger to water buffaloes from metal fragments, and to the farmer from unexploded bombs and shells. Consequently agricultural land quickly becomes overrun with weeds and bamboo, making reclamation even more difficult. About 10 per cent of the agricultural land of South Vietnam has been abandoned because of the ravages of the war.

Bringing Rothschild Down to Earth

This commentary on the Rothschild and Dainton reports is by Sir Frederick Bawden, Director, Rothamsted Agricultural Station.

ONE of the more extraordinary, and certainly more complacent, of the comments I have seen or heard on the Rothschild Report occurs at the end of Professor Swann's recent article in *Nature* (234, 379; 1971). Here he tells us not to worry, for Lord Rothschild's style of writing and concepts were as crude thirty years ago as they are today, but the end results were then good because, as Swann modestly admits, he rewrote the papers. Apparently nostalgia for what he calls the hilarious times when he and Lord Rothschild did research together on spermatozoa (whether basic or applied research, I know not, but I assume certainly not strategic) has overcome his usual commonsense. Does he not appreciate that the Rothschild Report has been published, that Swann is not going to rewrite it, and the government seemingly has accepted the customer-contractor principle enunciated by Lord Rothschild with almost fanatical zeal?

Apparently, too, benefit accrues to other things than Rothschild's writing by association with Professor Swann. For instance, the MRC and SRC, on both of which he has served, are "deeply impressive and are envied and imitated the world over. These structures must not be dismantled." Let me tell him that these things also apply to the ARC and NERC, which seemingly he is willing to dismantle as a sop to Lord Rothschild. But if he doubts it, they too could be made sacrosanct by appointing him a member, which would be much cheaper and more beneficial than adopting Lord Rothschild's recommendation for these councils. And how did he manage to serve on the MRC without knowing it has institutes as large as some financed by the ARC, which he says "simply ask to be taken over by the ministries"?

The Editor of *Nature* is less complacent than Professor Swann. He suggests that only one activity of the ARC, plant breeding, should be handed over to the Ministry of Agriculture, Fisheries and Food (MAFF). "To do more would be folly, to do less churlish". To which I can only reply, then let us be churlish. Does the Editor not appreciate that, by its very nature, this is a long-term activity, even with annual crops, and hence does not qualify for a contract but could be conducted only

with the 10 per cent surcharge that is proposed should be allowed for basic and long-term research?

By a curious coincidence, however, when on the several recent occasions I have asked the permanent secretary of the MAFF what research he requires, it has been one in plant breeding—"a hard wheat". We can forget for the moment that such wheats have already been produced as only one of the many products from a general research programme of wheat breeding adaptable to meet any requirements, and simply consider setting up contract terms. First, how hard is hard? Then, with our fickle climate and different weather in different places, what amount of variation in hardness is acceptable? What is the minimum acceptable difference in yield from soft wheats? What degree of resistance to lodging and to various pests and diseases is required? How long should the contract continue? How much should be spent on it? If the final variety should yield less than soft wheats, as is probable, will the farmer be subsidized to grow it? Again, perhaps, not to worry, the proposed Chief Scientist or Controller of Research and Development will know all the answers. And, if they do, no matter whether you think their requirements cannot be met, you must accept the contract, even though it may conflict with another statement in the report, that development work, which is what producing and multiplying a new variety is, should have a better than 90 per cent chance of success.

Perhaps some scientist other than Lord Rothschild may also be sure where to draw the line between basic and applied research. I don't know; all I know is that I have never met him. Much of plant breeding, and much work in other agricultural subjects, such as nutrition, physiology and pathology, falls between his extreme categories, and is of the kind called strategic science in the Dainton Report. It leads to a steady accretion of knowledge that is applied as it is gained. Eroding this kind of work would be parlous, for out of it has come the major advances that have so greatly increased agricultural productivity. Apparently, those of us, and there are many, who have worked in such subjects and been of some practical use were sinning, for Lord Roth-

schild says this work had no customer and "This is wrong". Only those who are well versed in such subjects and have practical knowledge of farming conditions do in fact know where research is needed and where it can help, in spite of Lord Rothschild's statement that: "However distinguished, intelligent and practical scientists may be, they cannot be so well qualified to decide what the needs of the nation are, and their priorities, as those responsible for ensuring that these needs are met." Who those responsible people are we are not told, but presumably the Chief Scientist will not be among them, unless perhaps he is undistinguished, unintelligent or unpractical.

Lord Rothschild produces no evidence of need for the changes he proposes, either of inefficiency of the existing systems or of substantial improvements to be expected. Has contract research by government departments been so economical or rewarding that the activity should be extended to ministries such as MAFF, which is not a "consumer" of research in the same sense as the Ministry of Defence? Is the £6.2 million a year Lord Rothschild tells us the MAFF now spends on research more valuable to agriculture than the money administered by the ARC? Certainly there is no evidence for this in the list of those awarded the Research Medal of the Royal Agricultural Society of England, a yard-stick indicating "research work of benefit to agriculture" initiated in 1955, I think at the suggestion of Lord Rothschild when chairman of the ARC. If it is less productive, it would be a useful sum for the MAFF to offer on contract terms to the ARC.

In a speech at Long Ashton Research Station during his chairmanship he commented, seemingly with approval, on the quality of the people working for the agricultural research service, for he said it would be difficult to better them in any comparable organizations in this country or elsewhere. This will become much less difficult should his recommendations be accepted, for then it may well prove impossible to recruit or retain people of such quality. Yet, apparently, the quality of the service is to be put at risk for no other reason than that there must be no departure from the only true dogma, that applied research must be done on the consumer-contractor principle. Indeed, while reading this reiterated doctrine, it seems odd that it should be published as a part of a green paper; somehow, a little red book, perhaps called "The Sayings of Rothschild", would seem much more appropriate.

Lord Rothschild and the Institute of Hydrology

A recommendation of the Rothschild report is that "the Institute of Hydrology should be physically transferred, together with its staff to the Department of the Environment where it should be fused with the DOE's contiguous Hydraulics Research Station". In this article, Dr. J. F. G. McCullouch, Director of the Institute of Hydrology, offers his opinion on this statement.

HYDROLOGY achieved formal government recognition in Britain when it was identified as one of the natural sciences, the development of which was entrusted to the Natural Environment Research Council set up in 1965. NERC inherited the Hydrological Research Unit which then comprised some eight individuals based in the first instance at the Hydraulics Research Station at Wallingford, Berkshire, as well as half of the Water Division of the Geological Survey and Museum, the other half having been absorbed into the Water Resources Board. Of course, other departments or agencies had interests in, and requirements for, hydrological expertise and in the absence before 1965 of a focus for hydrology, many of these organizations had set up small hydrological units to obtain generally *ad hoc* answers to particular problems. By 1971, however, the national situation regarding hydrology had changed completely. Under NERC, the Hydrological Research Unit had been expanded into the Institute of Hydrology with a staff of 95. This group, in close association with the Institute of Geological Sciences Hydrogeological Department, with a mainly geological staff of 36, possesses a formidable interdisciplinary competence to tackle any problem of hydrological research in any one or all phases of the hydrological cycle.

Hydrology is a practice which has of necessity been applied before the scientific principles have been fully understood. Unavoidably, solutions to particular problems have been given without the basic knowledge of the functioning of hydrological processes and with only a qualitative understanding of the operation of hydrological systems. Fundamental problems have been by-passed in favour of assumptions which are adequate as design criteria only if a large enough margin of error can be tolerated. How does rainwater reach the stream? What are the factors controlling evaporation from a forest and how do they differ from those affecting grassland or agricultural crops? How can point precipitation be measured to a known degree of accuracy? Such research is inevitably long-

term and interdisciplinary; the research hydrologist must be able to stand back, unharassed by day to day crises, the better to identify the gaps in the data, and indeed in the theory, available to the engineer. The flexibility of research council support for such research topics makes the existing British structure the envy of many other countries. For those who wish to work within the framework of a university department, research grants and contracts are negotiable; something of this atmosphere of imaginative endeavour permeates the institutes of the research councils.

The programme of research of the Institute of Hydrology may be divided into two parts. The first explores hydrological systems within complete catchment areas in studies of the influences of geomorphology, of geology and of land use on the response of particular drainage basins to rainfall. These studies have necessitated the instrumentation of a number of carefully selected small river basins in different parts of the country. Special instruments and techniques have had to be developed for assessment of such factors as the run-off in steep streams, soil moisture storage and movement, meteorological variables controlling evaporation and rainfall in remote areas. No fundamental scientific competence is claimed for the operation of such experimental catchment projects yet it is on the quality and continuity of the resulting data that the subsequent mathematical modelling of catchment behaviour depends. These models are based on the physical laws of water movement and permit derivation of future flows during flood and drought conditions and of their frequency of occurrence. They will provide the improvements to methods of forecasting and prediction that are vital for better resources evaluation.

Examination of whole catchments may provide knowledge of their gross responses as "lumped systems" but little understanding of the reasons why they function as they do, and the second comprises detailed studies of the component processes of the hydrological

cycle. The institute has also undertaken, stimulated by the Institution of Civil Engineers, a £250,000 3-year project which could from the outset have been contractable within the meaning of the Rothschild recommendation. This is the UK Flood Study in which a team of fifteen engineers and scientists is collating and analysing all available river flow and related data in Britain to produce a report on the magnitude and frequency of flooding. This report will be of fundamental importance to river engineers and others concerned in the design of flood control structures and bridges and is of particular relevance to the MAFF which carries a statutory responsibility for flood control and drainage; the speed, competence and economy with which the project was launched testifies to the flexibility of the research council structure.

The recommendation that the Institute of Hydrology be transferred to the Department of the Environment seems "exceptionally" at odds with the remainder of the Rothschild report. The Institute of Hydrology acts as a focus for basic hydrological science which is highly regarded both in Britain and overseas. At home, the programme of the institute fulfils a requirement identified by numerous committees, both of the old Department of Scientific and Industrial Research and of NERC, on all of which appropriate executive departments were represented.

As part of the British contribution to the International Hydrological Decade, NERC produced 18 months ago a detailed study of hydrological research in Britain within government, quasi-government and university departments. This excellent testimony to NERC's coordinating function revealed an apparent proliferation of water-based research, much of it so applied that it hardly merits being described as hydrological research. Whereas this might justify amalgamation into the DOE of all operational and development research connected with water problems from "source to sewer" it also enhances the need to sustain the national capability for advancing hydrological science within the research council system where it has been developed so successfully. Notwithstanding the Rothschild report, hydrology is as much an environmental science as oceanography and geology.

The Rothschild report also recommends that the Nature Conservancy, now a part of the Natural Environment Research Council (NERC), should be transferred as a whole to the Department of the Environment.

NEWS AND VIEWS

Viruses and Breast Cancer

At a press conference held at the US National Academy of Sciences last October (a transcript of which has been published in *Science* (174, 679; 1971) together with deserved praise for the responsible way in which the conference was reported in the US press), Spiegelman said, "Look, if a woman has a familial history of breast cancer in her family and if she shows (milk) virus and if she was my sister, I would tell her not to nurse the child". On page 32 of this issue of *Nature* Axel, Schlom and Spiegelman report some of the experimental evidence which no doubt led Spiegelman to his opinion.

Last February even the most cloyed of tumour virologists sat up and took notice when Moore and his colleagues (*Nature*, 229, 611; 1971) reported the discovery in certain human milks of a virus particle with a structure virtually identical with that of Bittner virus, an RNA tumour virus of mice which is transmitted in milk and which can cause mouse mammary carcinomas. Moreover, the distribution of this virus in the admittedly small sample of milks that were tested suggested that it might be more prevalent in the milk of women from families with a history of breast cancer than in milk from women in families without a history of the disease.

A few weeks later Schlom, Spiegelman and Moore (*Nature*, 231, 97; 1971) reported that human milk virus particles, like all the animal RNA tumour virus particles so far tested, contain the enzyme reverse transcriptase, which can use the single stranded RNA genome of a tumour virus as a template for the synthesis of a double stranded DNA containing the same genetic information. And as the biochemical and biophysical characterization of human milk virus progressed it became increasingly clear that this virus is best classified with the animal RNA tumour viruses and the mouse mammary carcinoma viruses in particular. Such was essentially the position by midsummer last year; human milk virus had very seriously to be considered as a possible causative agent of breast cancer because of its similarities to the murine viruses. But how can a causative association be proved? How can the possibility that this virus is only casually associated with breast cancer be eliminated, and if it is proved that the virus does indeed have an aetiological role what can be done to prevent or cure the disease?

Ask any microbiologist how definitive proof of the viral aetiology of a disease is obtained and he will surely reply that Koch's postulates, or some modern version of them, must be fulfilled. It must be shown that the suspected virus is encountered in every case of the disease and, most importantly, that pure preparations of the virus on injection cause the disease. Clearly this last test is out of the question for agents suspected of causing anything other than the most trivial human diseases and certainly the test can never be applied with agents suspected of causing cancer. Other less direct criteria have therefore to be fulfilled. For example, the virus can be tested for pathogenicity in lower primates and for human cells in culture; immunochemical evidence can be searched

for and attempts can be made to vaccinate against the disease using suitably inactivated preparations of the virus, and so on.

The first step therefore in trying to establish a causal relationship between a virus and a disease, between milk virus and breast cancer, is to look for evidence which fulfils Koch's first postulate. If it is assumed that human milk virus is indeed a human mammary tumour virus it should be possible to find evidence of the viral genome being expressed in human breast cancer tissue whereas in normal breast tissue there should be no evidence of virus replication. Translated into experimental terms, cancerous breast cells should contain RNA molecules identical to all or part of the RNA genome of milk virus whereas normal breast cells should not contain these RNA molecules. The results which Axel, Schlom and Spiegelman report on page 32 suggest that this condition may well be fulfilled.

Spiegelman's group has exploited the enzyme reverse transcriptase to make radioactively labelled DNA which has a base sequence complementary to the RNA genome in Bittner mouse mammary tumour virus. They were obliged to use the mouse virus rather than human milk virus simply because the latter is not yet available in quantities large enough for this type of experiment. In other words Spiegelman and his colleagues were obliged to gamble that the genomes of the mouse virus and human milk virus are very closely similar. In the event their gamble seems to have paid off handsomely, which, of course, only strengthens their belief that the milk virus might be closely related to Bittner virus—indeed is its human counterpart.

Having made radioactive DNA complementary to Bittner virus RNA Axel, Schlom and Spiegelman established that it could be used in molecular hybridization experiments to detect Bittner virus RNA in extracts of mouse mammary carcinomas. Their DNA is a probe specific for RNA with base sequences like those found in Bittner virus RNA. They then isolated the RNA from polyribosomal extracts from human malignant breast cancers, from benign human breast tumours, from normal human breast and from a few other human cancer tissues, and assayed the extent to which these various RNAs hybridize with Bittner virus DNA. Such experiments push the RNA/DNA hybridization technique to the very limits of its resolution, and Spiegelman's group, well aware of this and of the need to show that the hybridization they measure is specific, also tested their various human RNA samples for hybridization with DNA complementary to the RNA genome of a strain of mouse leukaemia virus.

Their results proved to be gratifying; of the twenty-nine RNA samples from malignant breast tumours nineteen (67 per cent) hybridized to mouse mammary tumour virus DNA to a significant extent. By contrast none of the forty RNA samples from the control breast and other tissues hybridized to a significant extent with

this DNA. Furthermore, and most impressively, Spiegelman's group are able to report that the RNA from cancerous breast did not hybridize with mouse leukaemia virus DNA. This result is expected, for there is ample evidence to suggest that the mouse mammary carcinoma viruses are quite unrelated to the mouse leukaemia viruses.

In short these data imply that among the RNA molecules in human breast cancer cells are sequences specifically complementary to mouse mammary tumour virus DNA, the implication being that these RNA molecules appear in the cancer cells because human milk virus is replicating in them. As Spiegelman and his colleagues are at pains to point out, however, these findings by no means prove that human breast cancer virus causes breast cancer. They do not in any way rule out the possibility that the virus replicates in the cancer cells because they are cancerous rather than the converse. But what Axel *et al.* report is entirely consonant with the hypothesis that human milk virus is a counterpart of Bittner virus, which is no doubt why Spiegelman was prepared to say that women producing milk virus might be well advised not to suckle their babies. Certainly with the provisos that he attached to his statement it is surely no more than most people would make just to play safe.

Whether it is dangerous to breast-feed babies if the milk they receive contains milk virus depends obviously, of course, not only on proving that the virus may be a necessary if not sufficient causative factor of breast cancer but also on establishing its routes of transmission. Although the Bittner virus is undoubtedly transmitted in milk, other types of mouse mammary tumour virus are, according to the Dutch school of RNA tumour virologists in particular, transmitted in the egg and sperm. Indeed there is some evidence to suggest that all mice may inherit the genetic potential to make a mammary tumour virus but whether they ever express this potential and/or contract mammary carcinoma seems to depend on additional genetic and hormonal factors. The crucial question, then, is simply whether the human milk virus is homologous to the Bittner agent or whether it is transmitted in egg and sperm as well as in milk. Bittner first established the milk transmission of the virus which bears his name by fostering experiments. Unwittingly a similar experiment may already have been done with humans. If it were possible to identify a population of women, who for one reason or another as babies received neither milk nor colostrum from their mothers, it might perhaps be possible to obtain for women data comparable to those Bittner produced for mice. If milk virus is crucially involved in the aetiology of breast cancer and transmitted via milk exclusively the incidence of breast cancer and of milk virus should be dramatically lower among such women than among a matched population of breast fed women.

What little evidence there is at present available suggests if anything that if a virus is involved in human breast cancer its transmission in milk may not be all that important. As Dr P. Shubik, for example, commented at last year's press conference (*Science*, **174**, 680; 1971), "there has been a huge decrease in breast feeding over the past 30 or 40 years and if anything there has been a slight increase in breast cancer" and, more to the point, Macklin for one (*J. Nat. Cancer Inst.*, **22**, 927; 1959) has published epidemiological data which suggest that in families the tendency towards breast cancer is inherited

equally through paternal as well as maternal lines. This observation, which merits close re-scrutiny and amplification, is not easily squared with the picture of a disease caused by an agent transmitted in milk. It is perhaps asking too much to expect that human breast cancer will follow precisely the Bittner virus pattern and that the incidence of the disease can be drastically reduced simply by preventing certain mothers from suckling their babies in the way that the incidence of mammary carcinoma among C3H mice can be cut simply by fostering on C57 black mothers.

If as might be the case all women inherit vertically the potential to produce a mammary tumour virus and develop breast cancer, whether any individual woman actually realizes this potential might well turn out to depend on other genetic factors, on her hormonal status and even perhaps on environmental and dietary factors. But while the epidemiologists are pursuing whatever leads they can find which may result in the elucidation of the pattern of incidence and inheritance of breast cancer, virologists will no doubt try to put their convictions to the test by first obtaining milk virus in useful amounts and then, by feeding it to monkeys or working with cells in culture, seeing if it has an oncogenic potential. At the same time they will doubtless attempt to devise cheap but reliable assays for the virus itself or for immunochemical markers of its impending production, assuming they exist. In spite of all the doubts and in spite of the absence of anything approaching a watertight case against human milk virus, it nevertheless remains a more plausible candidate for the distinction of being the first human cancer virus to be identified than any of the other RNA viruses which have been discussed in that context.

Tachyons and Gravitons

TACHYONS, like quarks and magnetic monopoles, are part of that growing list of physical objects that stand as a tribute to the resourcefulness of the theoretician. Such objects ought to exist if nature shares man's aesthetic tastes for mathematical symmetry, but so far nobody has actually succeeded in finding them. Nevertheless, historical examples indicate that if a physical possibility is compatible with the laws of physics as they are known, then nature tends to realize this possibility. When Dirac discovered the negative energy solutions to his famous equation, nature turned up the corresponding object in the form of the positron.

The case for the tachyon emerges from the special theory of relativity. Everybody knows that this theory prohibits the translation of material objects faster than the free space velocity of light. But this assumes that "ordinary" particles (called tardyons in this sort of discussion) have a mass m such that m^2 is greater than zero. If $m^2=0$ then the particles travel at the speed of light (for example, photons, neutrinos, gravitons, which can be called "luminons"). But what if m^2 is less than zero? This implies imaginary mass particles, but this is no difficulty because mass can never be measured directly anyway. Such particles have the bizarre property that they can only travel faster than light, and by so doing apparently violate the laws of causality, providing man with the possibility of signalling to his own past. Such unpalatable implications have not deterred a spate of

experimental and theoretical investigations of these strange objects, and a further contribution has been made by A. S. Lapedes and K. C. Jacobs of the University of Virginia in the January 3 issue of *Nature Physical Science* (235, 6; 1972).

The worlds of the tachyons and tardyons meet at the luminons, which hopefully interact with both, so that the natural way for tardyon beings to search for tachyons is by examining processes in which luminons are produced by the first and detected by the second. Photons are obvious candidates for this role, and one such process might be through what is known as Čerenkov radiation. This radiation may be thought of as an electromagnetic shock wave (rather like the bow wave of a ship) emanating from a charged particle which travels with a speed in excess of the light speed in that particular medium, just as a supersonic aircraft produces a sonic boom when it travels faster than the sound speed in air. Of course, Čerenkov radiation cannot be produced in free space by charged tardyons, because they can only travel at less than the vacuum light speed, but in a material medium the light speed is slower than in the vacuum, and it is in such a medium that Čerenkov radiation is observed. But for charged tachyons, the phenomenon should occur even in free space. The trouble is that a tachyonic electron would radiate all its energy in this way in about 10^{-19} seconds. A neutral tachyon would not be subject to this electromagnetic dissipation, however, but would still undergo an analogous gravitational Čerenkov effect that is a factor of some 10^{33} times less transient for a tachyonic neutron, giving it a "lifetime" of about 2 million years.

A formula for the radiation rate of these Čerenkov gravitons (a graviton is a quantum of the gravitational field) is derived by Lapedes and Jacobs. Their method is quite straightforward, being a direct analogy with the electromagnetic theory. Just as a charge can be the source of electromagnetic waves, so the stress-energy-momentum tensor can be the source of gravitational waves and, for weak fields, the expressions may be taken over more or less intact. The result is, as expected, a formula for the rate of energy dissipation which is similar to the corresponding electromagnetic formula, apart from the all important factor of 10^{33} . Inspection of the

formula shows a characteristic "decay" time which is proportional to m^{-3} . The authors also point out that if a tachyon has zero energy (called a transcendent tachyon) then the radiation rate is zero. This is in contradistinction to an earlier conclusion reached by L. S. Schulman.

The two chief questions in this subject have always been how can one detect tachyons and where does one look for them? If the possibility of these gravitational "booms" is accepted, one might expect to detect them using apparatus similar to

Weber's famous gravitational wave detector. Unfortunately, it seems that only cosmic sources could provide sufficient energy to make the detection of such booms a possibility using present techniques, but Lapedes and Jacobs do suggest (perhaps not too seriously) that tachyon objects (stars?) may reside at, or be produced in, the galactic centre, and be contributing pulses of gravitational radiation. Failing this, the ever fruitful big-bang origin of the universe is always a good place to produce anything, tachyons included.

FRESHWATER FISH

Revision of North American Chubs

from a Correspondent

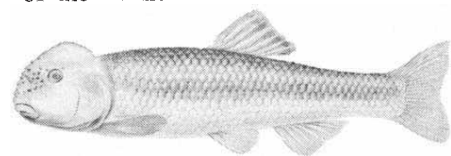
THE carp family (Cyprinidae) is one of the most successful groups of living fishes, and is the dominant element in freshwater fish faunas of the northern hemisphere. In northern America the cyprinids attain a degree of diversity which is probably unsurpassed elsewhere, and the profusion of species shows a striking degree of ecological variation without departing too greatly from the basic morphological framework of the group. Not surprisingly, there has been considerable confusion in the past between closely related species, and also failure to recognize polytypic species groups, not least in the genus *Nocomis*, popularly known as chubs in the eastern States. That this confusion has been long standing is illustrated by two recent articles by Ernest A. Lachner and Robert E. Jenkins (*Smithson. Contr. Zool.*, No. 85, 1; and No. 91, 1; 1971) in which they describe three previously unrecognized species from the eastern United States.

The first article is a review of the systematics, distribution, and evolution of the genus *Nocomis* of which previously three widely distributed and well differentiated species were known. Studies over a number of years, however, showed that these species are in fact species groups rather than clearcut taxa in their own right, and each contains three forms.

One feature which Lachner and Jenkins have used with considerable success has been the careful mapping of the abundance and pattern of head tubercles, a feature common to all cyprinids. These tubercles first appear in juveniles of between 30 and 80 mm body length as small round, light to grey areas in the deeper epidermis; in females they often remain at this stage of development, although in some species females develop a more distinct spot. In males just before the breeding season the tubercles are developed into

prominent, pearl tinted pimples, both on the head and the pectoral fins. They are deciduous after spawning, but leave recognizable scar tissue. Lachner and Jenkins find that the distribution, number, size and developmental patterns of the head tubercles represent the most important critical characters in the separation of the species of *Nocomis*. Using these characters, and more traditional criteria, Lachner and Jenkins have revealed a most interesting pattern of distribution within the three species groups.

In their second article Lachner and Jenkins consider the *N. biguttatus* group, where a similar distributional pattern emerges with the nominate species widely distributed through the north central United States, and the two recently recognized species have restricted distributions. *N. asper* has a centre of distribution in the Ozark highland part of the Arkansas River drainage, and *N. effusus* is found in the Green and Cumberland branches of the Ohio River and the Duck system of the Tennessee.



Crested, nuptial male *Nocomis microgogon* (from *Smithson. Contr. Zool.*, No. 85, 47; 1971).

The chubs of the genus *Nocomis* are moderately large fish, some species growing to 10 inches long, but they had formerly been little studied because they inhabit moderately deep, swiftly running rivers. Lachner and Jenkins's studies show the value of careful revisionary work to the proper understanding of the systematics of this genus—basic data for the appreciation of the distributional history of the freshwater fish fauna in the eastern states of North America.

SEISMOLOGY

New Array

from our Geophysics Correspondent

IN 1964 and 1965, the United States Government, seeing a political interest in extending the Partial Test Ban to cover underground explosions as well, installed the Large Aperture Seismic Array (LASA) in Montana. This array, which contains 525 short period and 63 long period seismometers, was thought of as a prototype for a world network of perhaps ten arrays which could provide adequate policing of an underground test ban. In the period since LASA was installed the interest in a test ban has waned, and with it the prospects for a network of LASAs. And yet it was clear from the very beginning that at least one more array would make sense scientifically. In fact, two more have been built—one in Norway called NORSAR and one in Alaska called ALPA.

The Alaskan array contains only long period seismometers, whereas NORSAR is similar to but somewhat smaller than LASA. The rationale for a second and third array runs like this: LASA reports a large number of low magnitude events on the basis of its short period recordings for which there is no adequate confirmation from any other station. Thus a second short period array of comparable power would provide a valuable cross check. The two arrays together could produce a bulletin of high credibility. On the other hand it has recently become increasingly apparent that the long period Rayleigh wave is crucial to identification of explosions. Detection of this is best done as close to the source as possible (this is not true for short period waves) and preferably on the same continent. With the United States interest in monitoring Eurasia, Norway was an obvious location for a long period array also, but in order to give increased azimuthal coverage an array in Alaska was also highly desirable.

A short conference was held in Oslo from November 22 to 24 to mark the opening of NORSAR. The first day was given over to demonstrations of the array's capabilities. Instrumentation at the 132 seismometer vaults over an aperture of 100 km is monitored and controlled from a central computer which also handles the recording of data and the real time forming of phased beams to hunt for events. 40,000 or so bits arrive every second. From the steady stream of tens of detected "events" per hour, a small number are finally accepted for the daily bulletin which reports ten or twenty events daily.

The two days of lectures were devoted to array and digital processing and the earthquake/explosion problem. K. Whitham (Ottawa) gave an invited talk

on identification of seismic events. He described recent research performed in his group on the surface wavebody wave criterion, short period discriminants and the capabilities of actual and proposed networks. His sober and careful assessment deserves a wider audience—particularly in the United States—than a meeting can provide. I. P. Pasechnik (Moscow) gave a Russian view of array usage in another invited contribution. Although arrays have been used in the Soviet Union for teleseismic observations, their chief use is in the huge programme of Deep Seismic Sounding which has produced some remarkable results.

First results on NORSAR's capability were presented by E. S. Husebye (chief seismologist at NORSAR), C. P. Felix (IBM), T. W. Harley (Texas Instruments), H. Bungum (NORSAR), J. R. Filson (MIT, Lincoln) and R. T. Lacoss (MIT, Lincoln). It seemed from these speakers that NORSAR was working well and efficiently. Short period signals are remarkably rich in frequencies higher than one cycle per second (in strong contrast with experience at LASA). Long period signals and noise were discussed at some length. Noise sources seem to cover a surprisingly wide range of azimuths. The Atlantic is frequently dominant, with micro-seismic storms arriving from bad

weather regions, but a significant proportion of the energy of the 20 s period range comes from the same general direction as signals from Russian underground explosions.

Array location capabilities were the subject of considerable discussion. I. Noponen (Helsinki), R. M. Sheppard (MIT, Lincoln), and E. Husebye (NORSAR) took very different approaches to the problem of understanding the sources of mislocation and making allowances for them. Several speakers examined further uses of arrays. D. Doornbos (Utrecht) showed uses of the vespa process for extracting core phases information at NORSAR. D. Davies (MIT, Lincoln) announced the discovery of phase PKJKP at LASA, yielding a shear velocity in the inner core of 2.9 km s^{-1} .

The meeting was, by any standards, a success. It brought together a diversity of European seismologists for very wide-ranging discussions on arrays and their uses, and indicated that NORSAR is already beginning to take root in the seismological community. An array demands of the seismologist a fundamentally new way of thinking about his subject—it is certainly much more than one station of ultra-high gain. It is clear that there are many seismologists prepared to take up this challenge of new methods.

Why So Big ?

LARGE mRNA molecules are now all the vogue in tumour virology. The ball was set rolling by Darnell's group (*Proc. US Nat. Acad. Sci.*, **65**, 1089; 1970), who reported that the virus-specific mRNA extracted from SV40-transformed 3T3 cells was considerably larger than that expected of a single polycistronic transcript. There were several possible explanations: first, that the mRNA was a concatenate of several polycistronic transcripts of an SV40 genome; second, that the mRNA was the product of transcription from a multimeric form of the SV40 DNA within the transformed cell; and third, that the mRNA contained non-viral nucleotide sequences, presumably cellular in origin. Darnell's group went on to show that the large mRNA did indeed contain cellular sequences, and this seemed to confirm that the SV40 genome is integrated into the host DNA in the transformed cell.

Evidence was then reported (Tonegawa *et al.*, *Cold Spring Harbor Symp. Quant. Biol.*, **25**, 823; 1970) to indicate that some, at least, of the virus-specific mRNA produced in cells lytically infected by tumour viruses is also too large to be the product of a single round of polycistronic transcription. Again, the several explanations

were available, and an attempt to distinguish between these alternatives is reported in next Wednesday's *Nature New Biology* (January 12). In this report, Jaenisch has examined the RNA: DNA hybrids made by annealing mRNA extracted from SV40-infected monkey kidney cells with SV40 DNA. He finds that although most of the virus-specific mRNA of size $6-10 \times 10^5$ daltons (the SV40 genome is about 2.5×10^6 daltons) forms hybrids which show high resistance to ribonuclease, the virus-specific mRNA which is of size $1.5-3 \times 10^6$ makes hybrids which are considerably more susceptible to RNAase digestion. Jaenisch argues, not unreasonably, that because the RNA is extensively degraded under the hybridization conditions used, this difference in RNAase susceptibility must reflect the presence of non-viral sequences in the large SV40 specific mRNA.

Some positive data are needed, of course, to show that the non-viral sequences are indeed cellular and not, say, poly A or other "nonsense". But the interpretation that they are indeed cellular is a reminder of the recent evidence of Defendi's group that the SV40 genome is integrated into the host DNA even during lytic infections.

PLANT PATHOLOGY

Disease Resistance

from a Correspondent

A DISCUSSION meeting on disease resistance in plants was held on December 9 at the Royal Society. Professor P. W. Brian (Cambridge), recalled in his opening remarks that the last meeting organized by the Royal Society on this subject had been in December 1947. The speakers on that occasion had been Professors W. Brown and F. T. Brooks, together with Mr F. C. Bawden. A link between these two meetings was provided by Sir Frederick Bawden as chairman of the afternoon session.

In his introduction Professor R. K. S. Wood (Imperial College, London) had time to say something more particular about the hypersensitive reaction of plants to invasion by bacterial and fungal pathogens and about the phytoalexin response. His former colleague at the Imperial College, Dr B. J. Deverall (Agricultural Research Council at Wye College), took up the story of phytoalexins in more detail. These protective substances are produced by plants in response to challenge by microorganisms and also to chemical injury; the possibility that phytoalexins can explain why most plants are resistant to invasion by most microorganisms accounts for the wide interest aroused by these plant chemicals. Weyerone acid has recently been identified by Dr Deverall and associates as a phytoalexin produced by the broad bean. From work with potato, the plant in which K. O. Müller first demonstrated the phytoalexin response, Dr D. D. Clarke (University of Glasgow) cited evidence for the view that virulent races of the potato-blight fungus inhibit the synthesis of phytoalexin by their host.

Professor A. Kelman and Dr L. Sequeira (University of Wisconsin, Madison), with a variety of examples, made the point that bacterial pathogens are a natural choice for the study of resistance responses by the host. Working with *Pseudomonas solanacearum*, which causes wilt of tobacco, they have shown that prior infiltration of leaves with heat-killed bacterial cells or their protein fractions will protect leaves later challenged by the living pathogen, and that this protective response can move out into untreated leaves. From studies on the virus-yellow complex of sugar beet, Dr G. E. Russell (Plant Breeding Institute, Trumpington) demonstrated the wide spectrum of resistance mechanisms against virus infection, including a useful degree of resistance to colonization of leaves by aphid vectors.

The increasing threat to wheat and barley from yellow-rust epidemics produced for Dr R. C. F. Macer (Rothwell

Plant Breeders Ltd, Lincoln) the most lively discussion of the day, though this did not detract from the promise of F_1 hybrid varieties as a new kind of answer to this problem. The last two papers in the afternoon served further to emphasize the central theme of this meeting, that is, the wide variety of disease-resistance mechanisms. Dr P. W. Talboys (East Malling Research Station) developed in detail his explanation of how the sequence of post-invasion events can account for the various responses of susceptibility, tolerance or resistance in plants invaded by vascular-wilt fungi. Similarly, Dr J. Rishbeth (University of Cambridge) was able to explain some resistance mechanisms of trees, such as the resin response of conifers, that are effective against root-infecting fungi.

The general impression left by this meeting was that few if any of the new developments in research described during the day were of secondary importance; to counter the genetic opportunism of evolving pathogens, plant pathologists would have to exploit to the full all the resistance mechanisms possessed by the plant.

TUMOUR VIROLOGY

Mitosis Required

from our Cell Biology Correspondent

ALTHOUGH nobody has yet suggested a good reason why it should be the case, many tumour virologists maintain that the transformation of cells by both RNA and DNA tumour viruses depends on the cell going through at least one round of division, a quantal mitosis, after it has been infected. The idea has an immaculate pedigree, stemming from the experiments of such people as Green, Todaro and Temin to mention but a few, and as Weiss now points out (*Virology*, **46**, 209; 1971) it ties in with investigations of the differentiation of myoblasts in culture which have led Holtzer and others to conclude that cellular transformation during normal differentiation depends on a quantal mitosis.

Most of the experiments with the RNA tumour viruses, which have been interpreted in favour of the quantal mitosis idea, have involved the use of drugs which inhibit either DNA replication or mitosis. What is notable about the

Variation in Chromosome Pairing in Diploid Wheat

BREAD wheat, *Triticum aestivum*, behaves as a classical allohexaploid ($2n=6x=42$) with regular bivalent formation at meiosis and disomic inheritance, although the three genomes, A, B, and D, are sufficiently homologous for chromosome pairing to occur between them. This homoeologous pairing is prevented by an allele at the *Ph* locus on chromosome 5B. All of the synthetic tetraploid and hexaploid wheats produced from the probable diploid ancestors show homoeologous pairing, indicating that none of the diploid plants used has an allele which prevents this. On the contrary, all plants of *Aegilops speltoides* that have been studied suppress the control by the *Ph* allele. *Aegilops speltoides* is considered the most likely contributor of the B genome. Although it has been assumed that control by the *Ph* allele originated in the polyploid wheats, the origin and establishment of this genetic control of chromosome pairing is still an unsolved problem.

In *Nature New Biology* next week (January 12), Dover and Riley report the first evidence of genetic variation, in a diploid species, which influences homoeologous pairing in polyploid wheat. This variation is present in *Aegilops mutica* which, like *Ae. speltoides*, has previously been shown to suppress the activity of the *Ph* allele. Homoeologous pairing and chiasma frequency were studied in several *T. aestivum* $\times$ *Ae. mutica* progenies. The tetraploid hybrids had the A, B, and D

genomes from a homozygous *T. aestivum* and the Mt genome from naturally heterozygous *Ae. mutica*.

Dover and Riley counted the chiasmata in seven hybrid families, and found that the chiasma frequencies for the individual plants fell into four classes with mean chiasma frequencies of 1.51, 4.13, 7.06 and 12.91 chiasmata per cell. In the lowest pairing class homoeologous pairing was prevented and there was a high frequency of univalents. In the highest pairing class bivalents, trivalents and quadrivalents were formed. The segregation into four pairing classes indicates that more than two alleles are involved, possibly two alleles at each of two loci. One family segregated 1:1:1:1, indicating heterozygosity at both loci in the parent plant.

The discovery in *Ae. mutica* of this variation in factors regulating chromosome pairing suggests that similar variation may be present in other diploid species. The effect of such variation in the diploids is not known, but if it is effective in hybrids lacking the *Ph* allele, it provides material for the immediate establishment of regular bivalent formation in the new spontaneous amphiploid, that is, a diploid with low pairing alleles would give a classical allopolyploid. The finding of this variation in a diploid provides evidence that the *Ph* allele need not have arisen at the tetraploid level. If similar variation exists in *Ae. speltoides* it would add weight to the evidence that this was the donor of the B genome of wheat.

experiments Weiss has just reported in *Virology* is the way in which he blocked DNA synthesis and mitosis of the infected cells. Instead of exposing chick cells to an inhibitor he plated freshly trypsinized chick cells, infected with Rous sarcoma virus, onto confluent monolayers of either mouse cells or chick cells. When mouse cell monolayers are used the chick cells do not replicate their DNA or divide, neither do they develop into foci of transformants. By contrast the infected chick cells plated on top of chick cell monolayers do divide and do develop into foci without the virus replicating in the monolayer cells.

Weiss believes that these findings are consonant with the quantal mitosis idea and he has an equally fashionable explanation for the differential inhibition of chick cell division by monolayers of chick and mouse cells. He suggests, *à la* Burger, that the mouse cells elaborate and secrete some cell surface component which is stripped from chick cells when they are exposed to trypsin and which is required to keep the cells susceptible to those growth regulatory signals which act in dense cultures to inhibit cell division. Freshly trypsinized chick cells plated onto a monolayer of mouse cells pick up this surface component and their multiplication becomes inhibited. By contrast, Weiss envisages that monolayers of chick cells do not secrete this component.

Be that as it may, Weiss is now hunting mutant Rous viruses which are conditionally sensitive to the density dependent growth of their hosts. Kawai and Hanafusa (*ibid.*, 470) who have also joined in the search for temperature sensitive mutants of Rous sarcoma virus have come up with one mutant *Ts* 68 which is more or less identical to the mutant isolated and described by Martin (*Nature*, **227**, 1021; 1970). Both the Martin and the Kuwai and Hanafusa mutants appear to define a Rous sarcoma virus gene which specifies a protein that is not a component of the virion but is required to maintain the transformed cell phenotype.

Progress towards the identification and isolation of this crucial viral protein will inevitably be tantalizingly slow, but analysis of the Rous virion proteins is progressing. Rifkin and Compans (*ibid.*, 485), for example, have now shown that the spikes, which, in the electron microscope, can be seen projecting from the surface of Rous virions, are removed by treatment with bromelain and their loss coincides with the loss of glucosamine containing proteins and of infectivity. Rifkin and Compans conclude therefore that the spikes of Rous sarcoma virions are glycoproteins necessary for infectivity and that they resemble the spikes of other enveloped viruses including flu and SV5 viruses.

PROTEIN SYNTHESIS

Asserting the Faith

from our Molecular Biology Correspondent

FROM time to time a need arises within each field of research to provide the faithful with a sign. Some fifteen years since the word concerning the triplet code went forth, Gupta *et al.* (*Biochemistry*, **10**, 4410; 1971) now offer more or less direct evidence that with each amino-acid incorporated into a polypeptide chain the ribosome does indeed, as everybody knew it would, slide, roll, bounce or lurch three nucleotide units along the messenger in the 5' to 3' direction.

Leaving out the separate processes of initiation and termination, the cycle of synthesis in bacterial systems is at present thought to go as follows: a quaternary complex is formed between a protein factor, GTP, an aminoacyl-tRNA and the A-binding site of the ribosome, sitting on its messenger, with the partly synthesized polypeptide chain, in the form of polypeptidyl-tRNA, at the other other (P) site. The GTP is then hydrolysed and the resulting GDP, still bound to the protein factor (S_2), as well as the orthophosphate that is formed, are liberated. The peptidyl-tRNA is hydrolysed, and the peptide chain makes a new peptide link with the amino-acid attached to its tRNA in the A-site. There is then a translocation step, evidently triggered by another ribosomal factor (S_3) with consumption of a

further GTP, at the end of which the peptidyl-tRNA has returned to the P-site, and the discharged tRNA has departed. Thereafter it is only necessary to regenerate a quaternary complex, a process which requires the intervention of a third factor (S_1) to dissociate the S_3 -GDP, for the cycle to repeat. One takes it that GTP hydrolysis provides the mechanochemical urge to displace the ribosome along the messenger, but Gupta *et al.* point out that the implication of GTP at two points in the cycle leaves some doubt about the exact stage at which translocation occurs. They have accordingly examined the system frozen in turn at each state of the cycle.

The messenger is f2 phage RNA, and an initiation complex, involving ribosomes and formylmethionyl-tRNA, forms at the beginning of the coat protein cistron of the messenger. By adding or omitting the appropriate factors, S_1 , S_2 and S_3 , the system can be put into the pre- or post-translocation state. All of the messenger not in the ambit of the ribosome, and therefore protected by it, can then be trimmed away with nuclease. The remaining messenger can be dissociated from the ribosome, and its 3' terminal sequence determined. The messenger trapped, after the digestion, in the initiation or the pre-translocation complex ends with the phenylalanine codon, UUU, corresponding to the fourth residue in the coat protein after formylmethionine. After translocation an additional triplet on the 3' side is found to be protected. This is ACU,

Helium in Globular Cluster Stars

GLOBULAR clusters are of great importance because they are the oldest stellar systems in our galaxy. Thus, their ages may not only set a limit on the age of the galaxy, but may also provide an important clue to time scale over which the universe as a whole has evolved. A great deal of careful observation is necessary before such clusters can be accurately dated and determinations made of the distance to a cluster (and hence absolute luminosities), the composition of the stars in the cluster (particularly their helium and "metal" content) and the point at which the cluster members are leaving the main sequence. These data can then be related to theoretical evolutionary tracks for stars near the main sequence.

One bone of contention arising from such studies has been the exact choice of helium content necessary to fit the theoretical models to the observed properties of particular clusters. In next Monday's *Nature Physical Science* (January 10) John Faulkner, of the Lick Observatory, points out a convenient method for determining this from the exact shape of the evolutionary track

followed by cluster members between the turn off from the main sequence and arrival at the giant branch.

Faulkner has looked at the best models now available for the cluster M 92. Between the main sequence and the giant branch, the rate of luminosity evolution of a star depends critically on its helium content, although in the main sequence and giant branch themselves composition is less important in this respect. This effect is largely independent of convection, the treatment of which is most uncertain in stellar evolution theory.

For M 92, Faulkner finds that comparison of the slope in the critical region with theoretical models of Simoda and Iben (*Astrophys. J. Supp.*, **22**, 81; 1970) implies a helium content of $Y=0.33$. Values as low as $Y=0.2$ can be specifically excluded. This method of determining helium abundance by accurate measurement of just one portion of the evolutionary track of a cluster, rather than trying to relate all of the observations to a complete theoretical model, offers a powerful aid in the study of globular clusters.

which codes for threonine, the next amino-acid in the coat protein sequence.

The identity of the messenger fragments in the initiation and pre-translocation complexes shows that there is no movement along the messenger up to this point. The pre- and post-translocation messenger fragments were then introduced into a cell-free system and allowed to direct synthesis of the corresponding oligopeptides, which were found indeed to be substantially the N-terminal penta- and hepta-peptide respectively of the coat protein. If either GTP or the factor, S_2 , is omitted from the reaction mixture, the movement along the messenger does not occur. Thus the position of the displacement step in the cycle, and the constituents that it specifically requires, are now pinpointed.

Sucrose-gradient centrifugation of the variously labelled complexes indicated that the pre-translocation complex sedimented marginally faster than either the initiation or post-translocation complexes, which were indistinguishable. This is tentatively interpreted in terms of a small deformation of the ribosome in the first state, relative to the other two. Gupta *et al.* argue that the cleavage of a single GTP molecule, which is evidently all that is involved in translocation, is likely to affect only a single protein, which, if it suffers a conformational change in the process, could well encompass the small hydrodynamic effect on the ribosome.

It should of course be recognized that interpretations of small changes in sedimentation characteristics, especially of potentially dissociable systems, have to be viewed with caution. The so-called 60S ribosomes, for example, which were reported to appear under certain conditions, can now be written off as a fragment. It has long been a commonplace of protein chemistry that subunit equilibria, such as dissociation into halves, manifest themselves in the ultracentrifuge as a single asymmetric boundary of intermediate sedimentation coefficient. If, as is common, the equilibrium is associated with a change in molar volume, an added characteristic will be a shift in the equilibrium, and therefore in the observed sedimentation coefficient, with rotor velocity and position in the cell, for on this depends the hydrostatic pressure generated in the cell at high gravitational fields. It was some time before the scales fell from the eyes of workers in the ribosome field, but in recent months, starting with Spirin and ending most recently with Van Diggelen *et al.* (*FEBS Lett.*, **19**, 115; 1971), the relevance of these facts to the ribosome situation was made apparent. Van Diggelen *et al.* have shown that *E. coli* subunits poised on the edge of association to 70S ribosomes respond in the expected manner to the change of

hydrostatic pressure down a centrifuge tube. In "derived" particles, containing an aminoacyl-tRNA, the 70S state is stabilized, and the effect is not observed.

RNA VIRUSES

Mapping Polio

from a Correspondent

DISCOVERING the gene order in the RNA viruses has not been an easy task. Because of the lack of genetic recombination the genome of the single stranded phages had to be partially sequenced before there could be any certainty about the answer. In the case of influenza virus, of course, its apparently easy recombination results from the fragmentation of the genome in the virion so that there was no gene order to discover.

Polio virus also presents its special problems. Although the viral RNA is all in one piece and there is a low level of recombination which has led to some tentative mapping, the map must be confirmed and its orientation with respect to the polio RNA is unknown. The entire genome is translated into a polycistronic protein which is then cut up into individual proteins and, apart from the problems this must pose for the viral economy, it means that the order of appearance of mature viral

proteins is no indication of their order of synthesis.

The classical method for examining the direction of synthesis of a protein requires a system which allows the completion of already initiated chains without the initiation of new ones. Until recently such a system (like the reticulocyte system for haemoglobin) was unavailable, but now Summers and Maizel (*Proc. US Nat. Acad. Sci.*, **68**, 2856; 1971) have described the use of the drug pactamycin to prevent initiation of polio translation. During the 20 minutes that it takes for polio RNA to be translated in the presence of the drug they were able to pulse-label with radioactive amino-acids and show that the capsid precursor molecule is labelled only if label is added early after drug treatment. This strongly suggests that the set of four capsid proteins contained in this precursor molecule (which represents the product of thirty per cent of the genome) is at the N-terminus of the polycistronic protein, and therefore, presumably, at the 5' end of the polio RNA.

The recombination map also found the capsid protein genes to be clustered, and there are hopeful signs that the use of shorter labelling times will increasingly refine the resolution of the method and allow the ordering of the four capsid proteins within the large precursor.

Degradation of Heterogeneous Nuclear RNA

WHEN animal cells are fed short pulses of radioactive precursors of RNA a large proportion of the precursor is incorporated in the nucleus into a heterogeneous population of RNA molecules which are rapidly degraded such that very little of this RNA accumulates in the cytoplasm. There is some evidence, obtained from investigations of the kinetics of labelling of heterogeneous nuclear and cytoplasmic polysomal messenger RNAs, which suggests that the former RNA may contain precursors to the latter but most of the heterogeneous RNA has a short half life. It has generally been assumed that the degradation of heterogeneous RNA occurs in the nucleus, but according to Aronson, whose investigation of this RNA in sea urchin embryos is published in *Nature New Biology* next week (January 12), the degradation may occur in the cytoplasm.

Aronson finds that label incorporated into heterogeneous nuclear RNA accumulates with time in cytoplasmic polyribosomes and in a cytoplasmic RNA fraction which sediments at 4S. This cytoplasmic 4S RNA is not transfer RNA and is too small to be a precursor to messenger RNA, and so Aronson concludes that it may be a degradation product of the heterogeneous nuclear

RNA. Clearly, if nuclear RNA is rapidly degraded to a population of 4S RNAs an endonuclease must be involved in the degradation process. Aronson therefore assayed extracts of whole cells, cell nuclei and cell cytoplasm for endonuclease activity using labelled heterogeneous nuclear RNA and synthetic RNAs as substrates. It is interesting that he found that crude cytoplasmic extracts, rather than nuclear extracts, contain about 98 per cent of the total recovered endonuclease activity. This enzyme degrades high molecular weight RNA to yield a 4S product but it does not attack the RNA in polysomes unless they have been exposed to EDTA. These properties make it a prime candidate for a role in RNA turnover and if it is truly a cytoplasmic enzyme, rather than a nuclear enzyme which leaks very readily indeed from the nucleus, it is hard to avoid the conclusion that most nuclear RNA is degraded in the cytoplasm.

Aronson suggests therefore that perhaps all the RNA made in the nucleus passes into the cytoplasm where it is degraded first to 4S RNA unless it associates with ribosomes and is protected from degradation. Further degradation of the 4S RNA might then occur within cytoplasmic lysosomes.

HERBICIDES

Potential Side-effects

from a Correspondent

THE potential side-effects of herbicides on soil microflora and fauna were the topic of a symposium organized by the Pesticides Group of the Society of Chemical Industry and held in London on November 29. One contribution related to the mesofauna; Mr W. Wilkinson (Jealott's Hill Research Station, ICI Ltd) stressed the difficulty involved in correlating the results of different workers investigating the same herbicide, a point taken up by a later speaker (Dr E. Grossbard, Weed Research Organization) with respect to the soil microflora. Adverse effects, said Mr Wilkinson, should be attributed in many cases to changes in vegetation rather than to direct toxic effects on the mesofauna. Long-term field trials with paraquat at Jealott's Hill showed the different reactions to cultivation of closely related species of cryptostigmatid mites.

Mr Wilkinson stressed the need for experimental standardization, and this view was supported by his colleague, Dr J. R. Anderson, who has worked on the effect of the bipyridylum herbicides, especially paraquat, on soil microflora. Using as parameters several microbial activities including enzymatic reactions, Dr Anderson concluded that the results indicated that microbial processes important in agriculture were not changed significantly, probably because paraquat is readily adsorbed on clay minerals. It is interesting that the actinomycetes are relatively sensitive to paraquat, probably as a result of the release of the herbicide from the decaying organic matter colonized by these organisms, the absence of any inhibitory effect by paraquat on the degradation of carbon-14 labelled plant material in soil and the fact that effects may tend to be more marked at normal rates than at rates ten times greater than normal.

Professor L. J. Audus (Bedford College, London) reported some studies on the effect of ioxynil and its octanoate ester derivative on the nitrification activity of *Nitrosomonas* and *Nitrobacter* and demonstrated that *Nitrobacter* is more sensitive than is *Nitrosomonas*. This is an important finding because in the case of other herbicides *Nitrosomonas* is the more susceptible organism. In pure culture experiments oxygen measurement with an oxygen electrode showed that the octanoate ester is twice as active in inhibiting nitrification as is ioxynil, but in soil perfluasate the situation is reversed, suggesting that the toxic ester may be converted back into ioxynil.

Dr Grossbard suggested that the

necessity for research on the effect of herbicides on soil microflora might be questioned because the concentrations required for an adverse effect are frequently well above those used in field application. She illustrated this with examples drawn from the Weed Research Organization and from the literature. This reassuring finding must not lead to complacency because (1) inhibitory effects can occur at field doses under certain conditions; (2) new chemicals, the performance of which cannot be foreseen, require testing; (3) transformation products (metabolites) in the soil may be more toxic than the parent herbicide; and (4) the concentration in contact with microorganisms in the soil may be higher than would be assumed from the concentration applied to the soil surface.

Mr J. A. P. Marsh (Weed Research Organization), in a joint paper with Dr Grossbard on substituted urea herbicides, provided other examples that microbial activities and numbers of microbial propagules are not inhibited by concentrations of less than ten or even a hundred times the normal dose.

Separate Big-bang for QSOs ?

THERE is a saying that cosmologists are fermions—no two cosmologists are in the same state at the same time. Nearly all cosmologists, however, agree that the universe is expanding. It was one of the extraordinarily happy coincidences in the history of science that redshifts nicely fitting the hypothesis that the galaxies are receding from us at a rate proportional to their distance were being measured at just the same time that general relativity was predicting that the universe should be unstable against a tendency to expand in that way. Unfortunately general relativity did not predict enough about the details of expansion and for several decades cosmology was a theoretical subject abounding in models of the universe, all of which fitted the facts.

This situation was altered by the discovery in the 1950s that certain objects which had been taken for stars had redshifts so large that, if the hypothesis is adopted that the redshifts reflect the expansion of the universe, the objects must be so far away that (given certain bounds on their size dictated by the rate at which their intensity fluctuates) the fact that they can be seen at all is unexplainable. Moreover, the statistical correlation between redshift and apparent brightness of these quasi-stellar objects is not consistent with the view of the universe derived from observations of galaxies, unless it is assumed that their source and distribution throughout the universe are radically different from those of normal galaxies.

To the many attempts to explain

The action of 3,4-dichloroaniline (DCA)—small amounts are detected when linuron decomposes—on the microflora is similar to that of linuron in some respects, suggesting that its effects are probably attributable to the parent material and not to the presumed metabolite DCA.

Mr P. Quilt (Weed Research Organization), Dr Grossbard and Mr S. J. L. Wright (Bath University of Technology) examined separately the constituents of the formulation of barban. The active ingredient is responsible for the inhibition of mineralization of nitrogen and glucose utilization but it enhances phosphatase activity. The solvent complex has either little effect or greatly stimulates certain activities. 3-Chloraniline found in barban-treated soil differs in some aspects from barban in its behaviour towards the soil microflora.

The general theme of the meeting was that more fundamental research along guidelines to be agreed was needed but at the present state of knowledge herbicides do not seem to present a serious hazard to the soil microflora and mesofauna.

these facts Le Floch and Lebreton have now added an extremely elegant and simple suggestion which is to be published in next Monday's *Nature Physical Science* (January 10). Their hypothesis is that quasars are taking part in an expansion from a big-bang of their own. This is not a profound physical theory; if it works it will not explain the facts but merely clarify which facts need explanation. They do not mention the physical explanation of the expansion but assume that in the flat space-time of special relativity normal galaxies and quasars are receding but not from the same source. And they assume that for both normal galaxies and quasars the expansion rate is proportional to distance. Their motivation for this is that it is known to be true for nearby galaxies and also that Mattig (*Astr. Nachr.*, **284**, 109; 1958) and Sandage (*Astrophys. J.*, **133**, 355; 1961) have shown that for a wide range of conditions only such expansion laws are satisfactory mathematically.

Le Floch and Lebreton give a geometrical construction the application of which to the observed luminosities and redshifts is capable in principle of determining where in space-time is the point from which the recession of quasars is taking place; that is, when and where the quasars' big-bang occurred. In their article they show that their simple hypothesis explains some of the puzzling features of the statistics of quasars, especially the uncommonly large luminosities of objects with large redshifts.

Research into Fish Diseases

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Although there have been many recent advances in research into fish diseases, some problems, for example, preventive medicine and the relationship between pollution and disease, remain to be investigated in detail.

MANY years ago J. Hughlings Jackson stated that "The study of the causes of things must be preceded by the study of things caused". Although this statement did not specifically refer to the study of fish diseases, it is a useful starting point for discussion. Apart from a few isolated and sporadic records in the older literature, the study of diseases of fish is a recent scientific preoccupation. The three earliest books which appeared during this century were German by Hofer¹, Roth² and Plehn³, and all are still much in evidence today. Schlumberger and Lucké wrote the first comprehensive review⁴ on tumours in fish, amphibia and reptiles, which has become a standard reference. Schlumberger is considered to be one of the founders of the renaissance of comparative pathology which is currently in evidence. The earlier teutonic interest still persists today and further useful information has been published in the past few years⁵⁻⁷. More recently documentation of the subject has appeared on both sides of the Atlantic⁸⁻¹², so that there is now a useful commentary on the current state of research into, and knowledge of, diseases of fish¹³.

Parasitic Diseases

Parasitic infestations have been recorded since classical times¹⁴, but any attempt to assess their taxonomy, life cycle and ecological effect in relation to the host is relatively recent. A survey of the modern literature shows that the parasitologist, initially, has been obsessed by the taxonomy of his subject and has paid little or no attention to the effects on the host, both local and general, the mechanisms of attachments to the host, the epidemiology, the immunological response of the host or the possibility of the parasite as a vector of virus disease. Although the taxonomy of parasites is important, it is now appreciated that a wider study of the effects of parasites on their hosts is of greater importance, economically and ecologically, and that the morphology of parasites in relation to their host may only be of marginal interest¹⁵⁻¹⁷.

Host-parasite relations are being investigated in far greater depth, using many modern veterinary and medical techniques^{18,19}. Hoffman has reported²⁰ work associated with the intercontinental and transcontinental dissemination and transfaunation of fish parasites, and has alerted governmental agencies to the dangers of fish importation, without the necessary control. This point is well illustrated in the case of whirling disease, an often fatal disease in the younger Salmonidae. This disease, which is caused by an infestation with *Myxosoma cerebralis*, has been well documented in Europe

since 1903, but was unknown in the United States until 1956. The strict control of fish imported from one country to another must be enforced if potential damage to indigenous species is to be prevented. Although the Salmonidae particularly have been mentioned as being affected adversely by "imported diseases", many other examples could be given although documentation is far from complete. The emphasis on the control of spread of parasites and their treatment must be based on the preventive aspects because, once a parasitic infestation is established, treatment is often impossible and impractical. Care must be taken not to import or export fish without a licence certifying that they are free from disease. Once fish have become infested, unless it is by ectoparasites, treatment can be aimed only at either breaking the life cycle or preventing re-infestation wherever possible. Systematic treatments have been singularly unsuccessful. The immunological aspects of parasitic infestation in fish still await further elucidation. This is a branch of the subject which is becoming more firmly established and may prove to be of value in parasitic control. Parasitic infestations can be used in a more positive way in that their presence or absence can be used as a "biological tag"—this type of approach has been used recently with flounders²¹.

Bacterial Diseases

Bacterial diseases of fish were first documented just before the turn of the century; the earliest reports were from the continent of Europe²²⁻²⁴. More recently, an extremely valuable bibliography, compiled by Conroy²⁵ and containing more than 800 references, gave some idea of the amount of research expended in this field. Furunculosis was, in all probability, the first infectious disease to be fully documented in Britain and a comprehensive report was published by Scottish workers in the middle 1930s²⁶. The current leaders in the field of fish microbiology, who include Snieszko⁹, Ghittino¹² and Reichenbach-Klinke^{6,7}, have been in this area of research for many years and have established methodologies and disease patterns for many of the more important bacterial infections.

The two notifiable fish diseases in England are furunculosis and columnaris disease. These conditions represent a curious link with the 1937 Act, and their notification seems no longer to be taken seriously. The research in this particular field, while steadily progressing, is dictated by the commercial implications of the infection, because it is often impracticable to investigate diseases which have either been ill defined and/or have not presented a significant threat to either cultured or free living species. The activity in this field is now being directed towards the prevention of disease rather than its therapy. Chemotherapy is important in the treatment of cultured or aquaria fish, but is often extremely impractical in open waters because of the cost of the therapy and the possible persistence of various compounds in the flesh of the fish. Some governmental agencies are strict about drug residues in fish and it is desirable that no therapy be given to fish for three weeks before marketing. One of the chief problems in fish microbiology has been the establishment of Kock's postulates; failure to do this has resulted in many abortive attempts to

establish an accurate diagnosis. Kock's postulates are not easily established by the amateur investigator and the interdisciplinary approach to the study of fish diseases cannot be over-emphasized.

The relatively recent outbreak in Britain of ulcerative dermal necrosis is a useful example of how disconcerting uncoordinated research can be. One of the principal difficulties is the establishment, beyond reasonable doubt, of whether the organism is an obligate or facultative pathogen, and whether it is a primary or secondary invader²⁷. Only careful and expert research can establish these facts. Fish tuberculosis is another extremely important infection in fish microbiology²⁸, because it presents different problems in different fish species, problems related to its immunological and antigenic aspects, to its adaptation to various temperature and salinity²⁹ ranges and its synergistic effect with other infections. These are but a few of the questions associated with this infection which require further investigation. *Vibrio* infections have over the years been investigated by many workers^{12,30,31}. This infection is known as redpest in eels and is seen in reared yellowtails (*Seriola quinqueradiates*) and rainbow trout (*Salmo gairdnerii*), and occasionally is referred to as ulcer disease because it may be associated with superficial ulcerations of the skin. Further work on vibrio infections is being performed by Conroy and his colleagues at the Zoological Society of London, in the newly formed Unit of Fish Pathology.

Viral Diseases

The earliest research into virus diseases of fish was made by Weissenberg in 1914³² who investigated "lymphocystic disease". To celebrate the first fifty years of viral research in fish, the New York Academy of Science held a symposium on virus diseases in poikilothermic vertebrates³³. Much of the recent work and the recent advances in fish virology has been due to Wolf who has reviewed the current situation^{34,35}.

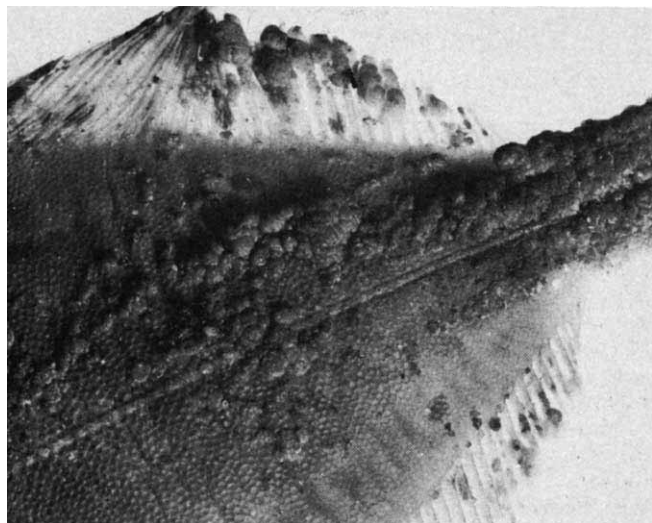


Fig. 1 Plaice, showing lymphocysts. This viral infection can easily be mistaken for a neoplastic condition.

Fish virology is still a relatively unexplored speciality and the possible viral aetiologies of many conditions have still to be established³⁶. Although fish virology has followed closely on the methodologies used in homeothermic vertebrates, it must be remembered that the study of poikilothermic vertebrates offers challenging differences. Of the significant advances in fish virology during the past few years the application of the virus plaque assay^{37,38} must be particularly mentioned. Of the diseases listed in Table 1, several should be noted as being of particular interest. The exact aetiology of infectious dropsy of carp (IDC) has caused much confusion. Fijan and his colleagues³⁹ have recently identified a new virus, *Rhabdovirus*

carpis, as being responsible for the acute form of IDC; the acute form is now called spring viraemia of carp (SVC) and the chronic form is referred to as carp erythrodermatitis (CE). Fijan has shown⁴⁰ that the two conditions have different aetiologies, epidemiologies and signs so that the term infectious dropsy of carp should be abandoned as further confusing an extremely difficult area of research. Mention must also be made of a condition the aetiology of which has still to be elucidated, but which may be considered to have a possible viral component—ulcerative dermal necrosis (UDN), a disease which affected first Irish salmon (1964) and then spread to many English and Scottish waters in subsequent years (1966 to 1969).

Table 1 Viral Diseases of Fish

RNA viruses	
Viral haemorrhagic septicaemia (VHS)	
Infectious pancreatic necrosis (IPN)	
Spring viraemia in carp (SVC)	
Carp erythrodermatitis (CE)	
Infectious haematopoietic necrosis virus (IHNV)	
Orphan virus or epitheliocystic disease	
DNA viruses	
Lymphocystic virus (LV) (Fig. 1)	
Channel catfish virus (CCV)	
Possible viral infections	
Ulcerative dermal necrosis (UDN)	
Papillomata of flatfish (Pleuronectidae)	
Lymphosarcoma (in pike)	
Carp pox	
Stomatopapillomata in eels (<i>Anguilla vulgaris</i>)	
Walleye neoplasms	
Malignant kidney tumour	
Grunt fin agent (GFA)	

Although Carbery and Strickland⁴¹ were the first to describe this condition, Roberts⁴²⁻⁴⁶ and his colleagues have performed extensive research into this disease and have documented in great detail many of the histological changes seen in salmon in the United Kingdom. These workers have so far not established a definitive aetiology but have considered many possibilities, including a primary CNS lesion which might be responsible for the trophic changes. Roberts has shown that once the skin lesion has become established, then secondary infection with *Saprolegnia* spp., *Aeromonas* or *Pseudomonas* spp. is not an uncommon occurrence and may often be the cause of death. It has also been noted that this disease is often associated with the transition of the salmon from a salt to a fresh water environment, and its associated stress. The significant physiological changes associated with this transition are mediated by the endocrine system. Such changes, together with some degree of stress, may well play a significant part in this multifactorial disease. Research is still being undertaken into this condition but it is reassuring to note that Roberts states "while the loss of many prime fish is bound to give rise to concern, it is not biologically disastrous".

Neoplastic Diseases

Although at first sight neoplastic change in fish may be thought an unlikely condition, increasing emphasis has been placed on this subject during the past twenty years^{4,10,47-49}. Neoplasms have been reported from many species of bony and cartilaginous fish and some are of particular interest to the epidemiologist because they are only seen in certain species in certain geographical locations. In the British Isles, the best example has been given by Mulcahy at Cork, who has reported⁵⁰ many pike (*Esox lucius*) suffering from lymphosarcoma. Many of these neoplasms, which have a definite geographical distribution, may be of viral origin, although in many instances this is still to be established. It would seem likely that viruses will ultimately be implicated in the papillomata of flatfish seen off the Pacific Coast of the North American continent, and in the stomato-papillomata seen in eels off the North European

coast. Much further research is required into the establishment of an exact aetiology for many of these neoplasms. It seems possible that these tumours will be ultimately associated with a known aetiology. Can they still be considered to be truly neoplastic? Of the many tumours reported from all types of tissue, it is the tumour of mesenchymal tissue which predominates in fish. This is in complete contrast to those seen in the higher vertebrates, including man, where the tumour of epithelial tissue is the most common. The subject of comparative neoplasia is an area for further research. The relationship between the lymphoreticular system and neoplasia requires much further investigation. The use of the fish in the study of hepatocarcinogenesis is often ignored as irrelevant by the cancer worker who has become accustomed to working only with rats and mice. The sensitivity of the rainbow trout to aflatoxins and nitrosamines has proved the value of such an animal model. Further basic investigations into the mechanism of hepatocarcinogenesis by aflatoxins are being made, together with a detailed biochemical study of the metabolic pathways in various fish species⁵¹.

Following the use of the more persistent biocides, much concern has been expressed in recent years as to their long term effects on wild species. The long term effects of sub-lethal doses of even DDT are ill defined and insufficiently documented and further study is required⁵². It has been shown recently that 0.013 p.p.m. of DDT in the water can significantly alter the amounts of various hepatic enzymes in carp (Barry, personal communication). Pollution as an aetiological agent in neoplasia is another interesting consideration. It has been known for some time that bottom feeding fish living in polluted waters have a high incidence of squamous cell carcinoma of the lip, snout and mouth and the possible link between these two factors cannot always be lightly dismissed, particularly when water residues have been shown to have a carcinogenic effect when painted onto the backs of mice. Much work is now being performed by the Comparative Oncology Committee of the Cancer Epidemiological Group of the Union International Contra Cancer (UICC) into assessing the amounts of pesticides found in fish and other marine creatures with tumours. It is hoped that this five year study will help to elucidate some of the problems possibly associated with pollution and neoplasia.

The Future

Research into fish diseases is not, as has been shown, solely the problem of the comparative pathologist, but rather of the fish biologist in general. Many current pressing problems need still to be solved, and none is more urgent than that of pollution. Lethal values are often well established, but these have little relevance in reality. What are the long term effects of sub-lethal amounts of pollution on fish and their disease; what is their exact *modus operandi*? Many of these man-made conditions have still to be investigated in detail.

In relation to disease processes, research must be continued into the various aspects of preventive medicine, so that with the help of the fisheries manager, the nutritionist, the geneticist and the immunologist, healthy fish will be raised, which will be resistant to the more common bacterial, viral and parasitic infections. Advances along all these lines have already been made, but much has still to be learnt.

- ⁸ Dawe, C. J., and Harshbarger, J. C. (eds.), *Neoplasms and Related Disorders of Invertebrates and Lower Vertebrate Animals* (Monograph 31, Nat. Cancer Inst., 1969).
- ⁹ Snieszko, S. F. (ed.), *A Symposium on Diseases of Fish and Shellfishes* (American Fisheries Society, Washington DC, 1970).
- ¹⁰ Mawdesley-Thomas, L. E. (ed.), *Diseases of Fish* (Symposium of the Zoological Society of London, No. 30) (Academic Press, London and New York, in the press, 1972).
- ¹¹ Amlacher, E., *Textbook of Fish Diseases* (transl. by Conroy, D. A., and Herman, R. L.) (TFH Publications Inc., Jersey City, 1970).
- ¹² Ghittino, P., *Pisciocoltura e Ittiopatologia*, 2; *Ittiopatologia* (Rivista di Zootechnia, 1970).
- ¹³ Neuhaus, O. W., and Halver, J. E. (eds.), *Fish in Research* (Academic Press, New York and London, 1969).
- ¹⁴ McGregor, E. A., *Spec. Scient. Rep. US Fish Wildl. Soc.*, No. 474 (1963).
- ¹⁵ Dogiel, V. A., Petrushevski, G. K., and Polyanski, Yu. I., *Parasitology of Fishes* (translated by Z. Kobata) (Oliver and Boyd, Edinburgh, 1961).
- ¹⁶ Hoffman, G. L., *Parasites of North American Freshwater Fishes* (University of California Press, Berkeley, 1967).
- ¹⁷ Sindermann, C. J., *Principal Diseases of Marine Fish and Shellfish* (Academic Press, New York and London, 1970).
- ¹⁸ Noble, E. R., and Collard, S. B., in *A Symposium on Diseases of Fishes and Shellfishes* (edit. by Snieszko, S. F.), 57 (American Fisheries Society, Washington DC, 1970).
- ¹⁹ Lee, F. O., and Cheng, T. C., *J. Fish. Biol.*, 2, 235 (1970).
- ²⁰ Hoffman, G. L., in *A Symposium on Diseases of Fishes and Shellfishes* (edit. by Snieszko, S. F.), 69 (American Fisheries Society, Washington DC, 1970).
- ²¹ Gibson, D. I., *J. Fish. Biol.*, 4 (in the press, 1972).
- ²² Canestrini, G., *Atti Ist. Veneto Sci.*, 4, 809 (1893).
- ²³ Emmerich, R., and Weibel, E., *Arch. Hyg. Bakt.*, 21, 1 (1894).
- ²⁴ Bataillon, E., Dubard and Terre, L., *CR Soc. Biol.*, 49, 446 (1897).
- ²⁵ Conroy, D. A., *Fish Biol. Tech. Pap.*, FAO, 73 (1968).
- ²⁶ Mackie, T. J., Arkwright, J. A., Pryce-Tannant, T. E., Mottram, J. C., Johnston, W. D., and Menzies, W. J. M., *Final Report of the Furunculosis Committee* (HMSO, Edinburgh, 1935).
- ²⁷ Snieszko, S. F., *Rev. Ind. Microbiol.*, 5, 97 (1964).
- ²⁸ Reichenbach-Klinke, H., in *Diseases of Fish* (edit. by Mawdesley-Thomas, L. E.) (Symposium of the Zoological Society of London, No. 30) (Academic Press, London and New York, in the press, 1972).
- ²⁹ Conroy, D. A., in *A Symposium on Diseases of Fishes and Shellfishes* (edit. by Snieszko, S. F.), 273 (American Fisheries Society, Washington DC, 1970).
- ³⁰ Rucker, R. R., *Fish Cult.*, 21, 22 (Prague, 1959).
- ³¹ Ross, A. J., Martin, J. E., and Bressler, V., *Bull. Off. Intern. Epizoot.*, 69, 1139 (1968).
- ³² Weissenberg, R., *Sitzungsber. Preuss. Akad. Wiss. Physik. Mathem.*, 30, 792 (1914).
- ³³ Snieszko, S. F., Nigrelli, R. F., and Wolf, K. (eds.), *Ann. NY Acad. Sci.*, 126, art. 1 (1965).
- ³⁴ Wolf, K., *Adv. Virus Res.*, 12, 35 (1966).
- ³⁵ Wolf, K., in *Diseases of Fish* (edit. by Mawdesley-Thomas, L. E.) (Symposium of the Zoological Society of London, No. 30) (Academic Press, London and New York, in the press, 1972).
- ³⁶ Schäperclaus, W., in *Handbuch der Virusinfektionen bei Fischen* (edit. by Rohrer, H.), 1067 (Gustav Fischer Verlag, Jena, 1969).
- ³⁷ de Kinkelin, P., and Scherrer, R., *Ann. Rech. Vet.*, 1, 17 (1970).
- ³⁸ Wolf, K., and Jorgensen, P. E. V., *Arch. Ges. Virusforsch.*, 29, 337 (1970).
- ³⁹ Fijan, N., Petrincic, Z., Sulimanović, D., and Zwillenberg, L. O., *Vet. Arhiv.*, 41, 125 (1971).
- ⁴⁰ Fijan, N., in *Diseases of Fish* (edit. by Mawdesley-Thomas, L. E.) (Symposium of the Zoological Society of London, No. 30) (Academic Press, London and New York, in the press, 1972).
- ⁴¹ Carbery, J. T., and Strickland, K. L., *Irish Vet. J.*, 22, 171 (1968).
- ⁴² Roberts, R. J., Shearer, W. M., Munro, A. L. S., and Elson, K. G. R., *J. Path.*, 97, 563 (1969).
- ⁴³ Roberts, R. J., *J. Fish Biol.*, 2, 223 (1970).
- ⁴⁴ Roberts, R. J., *J. Fish Biol.*, 2, 373 (1970).
- ⁴⁵ Roberts, R. J., Ball, H. T., Munro, A. L. S., and Shearer, W. M., *J. Fish. Biol.*, 3, 221 (1971).
- ⁴⁶ Roberts, R. J., Shearer, W. M., and Munro, A. L. S., *J. Fish. Biol.*, 4 (in the press).
- ⁴⁷ Wellings, S. R., in *Neoplasms and Related Disorders of Invertebrates and Lower Vertebrate Animals* (edit. by Dawe, C. J., and Harshbarger, J. C.) (Monograph 31, Nat. Cancer Inst., 1969).
- ⁴⁸ Mawdesley-Thomas, L. E., *J. Fish. Biol.*, 1, 187 (1969).
- ⁴⁹ Mawdesley-Thomas, L. E., in *Current Topics in Comparative Pathobiology* (edit. by Cheng, T. C.), 1, 87 (Academic Press, New York and London, 1971).
- ⁵⁰ Mulcahy, M. F., *Proc. Fourth Intern. Symp. Comp. Leukaemia Res.* (Bibliotheca Haematologica), 609 (S. Karger, Basle, 1970).
- ⁵¹ Mawdesley-Thomas, L. E., and Barry, D. H., *Nature*, 227, 738 (1970).
- ⁵² Mawdesley-Thomas, L. E., *New Scient.*, 49, 74 (1971).

¹ Hofer, B., *Handbuch der Fischkrankheiten* (Munich, 1904).

² Roth, W., *Die Krankheiten der Aquarienfische* (Franck'sche Verlagshandlung, Stuttgart, 1922).

³ Plehn, M., *Praktikum der Fischkrankheiten* E. Schweizerlartsche (Stuttgart, 1924).

⁴ Schlumberger, H. G., and Lucké, B., *Cancer Res.*, 8, 657 (1948).

⁵ Schäperclaus, W., *Fischkrankheiten* (Akademie Verlag, Berlin, 1954).

⁶ Reichenbach-Klinke, H., and Elkan, E., *The Principal Diseases of Lower Vertebrates* (Academic Press, London and New York, 1965).

⁷ Reichenbach-Klinke, H., *Krankheiten und Schädigungen der Fische* (Gustav Fischer, Stuttgart, 1966).

Continental Drift and the Dispersal and Evolution of Organisms

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Plate tectonics offers a framework in which aspects of the evolution and dispersal of organisms can be understood.

IN the past ten years continental drift has become firmly established, principally because of studies of the magnetic field at the surface of the ocean and of the distribution and mechanisms of earthquakes. As a result of this work the earlier concepts of continental drift and seafloor spreading¹ have given rise to an exact description of the surface motions of the Earth which is now known as the theory of plate tectonics^{2,3}. This theory, combined with magnetic observations, provides a method of reconstructing the position of continents at earlier times whose accuracy is as good as the navigation of the ships which observed the magnetic lineations—about 10 km. The date of the reconstruction can also be obtained with an error of less than one million years. It is therefore no longer profitable for biologists to speculate about the past arrangement of land masses. It is, however, of great interest to use the known positions of and connexions between continents obtained from the geophysical studies of the oceans to study the evolution and dispersal of land organisms; the purpose of this article is to show how this may be carried out, using the marsupials as the chief example.

The theory of plate tectonics which forms the basis of all reconstructions is remarkably simple. Its starting point is the observation that earthquakes occur principally in long linear bands⁴ surrounding large areas in which no shocks occur. As all earthquakes are the result of deformation it is reasonable to suppose that all deformation takes place in the seismically active belts and that none occurs within the aseismic regions. The aseismic regions are called plates, and it is now known that it is the relative motion between these plates which causes earthquakes. There are two principal differences between plate tectonics and the earlier ideas. The first is the division of the world into plates by the seismic boundaries: a single plate often contains both oceanic and continental regions, and the Earth's surface is entirely covered by a mosaic of plates in motion. The other important difference is the emphasis on relative, not absolute, motion. All phenomena at plate boundaries are due to the relative motion between the plates on either side and the concept of absolute motion is at present meaningless.

There are three types of plate boundary. Where two plates are moving apart, material from below wells up and cools, and hence adds to the plates on both sides. Such plate boundaries are commonly linear features and are called ridges. They are the sites of huge outpourings of lava and as the lava cools it becomes magnetized by the prevailing Earth's field. Because the main field of the Earth reverses periodically, ridges form long linear blocks of volcanic material magnetized in opposite directions. The magnetic anomalies formed by this process were first described by Vine and Matthews⁵ and provide the basis of all reconstructions in this article. Plates can also slide past each other without any plate generation or destruction on structures known as transform faults⁶, and they can be thrust beneath island arcs and destroyed as they sink back into

the mantle from which they were formed. The lines along which such plate destruction occurs are known as trenches.

The procedure by which magnetic anomalies are identified is adequately described elsewhere⁷⁻⁹. Distinctive anomalies have been numbered from 1 to 33 in order of increasing age. Thus any region of the seafloor on which, for instance, anomalies between numbers 1 and 20 can be identified must have been formed after the time when anomaly 20 was produced. It is therefore possible to reconstruct the positions of all plates at the time of anomaly 20 by removing all regions of seafloor on which anomalies from 1 to 20 (refs. 10-12) occur. In practice such reconstructions must be carried out by rotating plates through finite angles about axes passing through the centre of the Earth¹⁰. The reconstructions in this article were obtained from the observations of magnetic lineations of Pitman *et al.*⁷ in the Pacific, McKenzie and Sclater¹² in the Indian Ocean, Dickson *et al.*⁸ in the South Atlantic, and Pitman *et al.*¹³, Williams and McKenzie¹⁴ and Pitman and Talwani¹⁵ in the North Atlantic. The poles and rotation angles used were taken from refs. 10-12 and 15. Fig. 1 shows the present arrangement of the continents. Figs. 2-4 show their arrangements at -35, -45, and -75 million years (Ma), respectively.

Isolation and Adaptation

Since Darwin and Wallace, biologists have emphasized the crucial importance of geographical isolation in speciation and subsequent adaptation and differentiation of species. Many land animals and plants cannot cross an ocean, which therefore acts as a barrier to their dispersal. Thus continents act as boats whose passengers are organisms and during the period in which they are passengers evolution can take place. A simple rearrangement of landmasses will affect the evolution and dispersal of organisms. When two landmasses separate, the passengers of each become isolated from potential competitors which evolve on the other, so that they have a better chance to adapt and differentiate themselves to exploit their changing environment. When a land mass approaches and comes into contact with another the passengers of each can disembark, compete and differentiate in the territory of the other.

For example, before -43 Ma Australia, Antarctica and South America formed a single land mass. Marsupials probably reached Australia from South America by way of Antarctica. At -43 Ma Australia separated from Antarctica and moved northwards. Its marsupials differentiated and specialized, being immune to competition from all placental mammals (except bats) which evolved elsewhere. In the past 10 Ma or so, as Australia approached the Indonesian islands, there has been a limited exchange of mammals. Small rodents (murids) have reached Australia from the north, have competed successfully and have undergone considerable differentiation. A few small rodent-like marsupials have spread to islands near New Guinea where they have also undergone some slight differentiation¹⁶. Fig. 5 shows our reconstruction of marsupial evolution and dispersal.

We now consider the evidence for this reconstruction. Today native marsupials are found in Australia (and nearby islands) and in North and South America. There are about 170 living Australian marsupial species²⁵. They display many

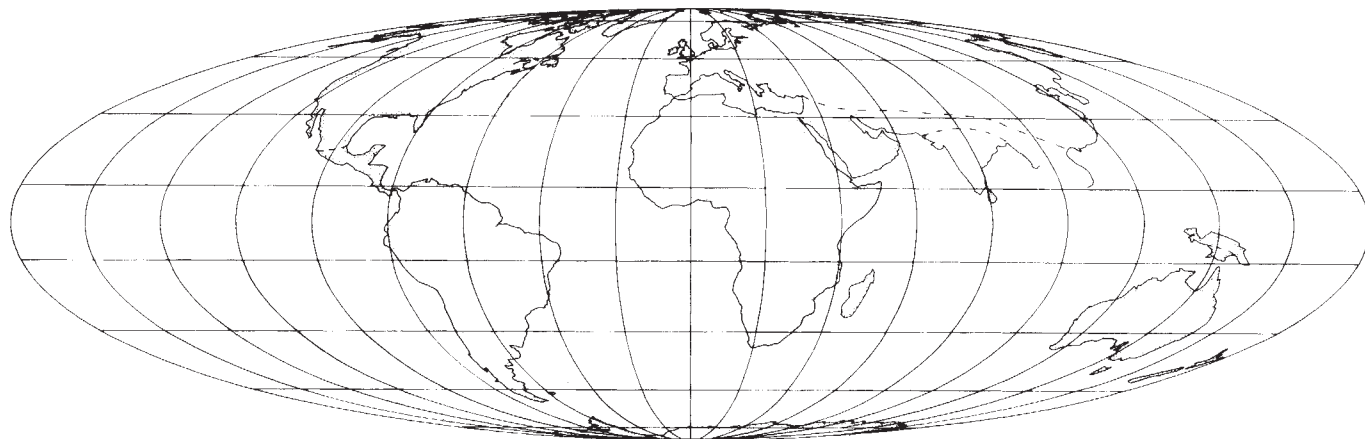


Fig. 1 Present arrangement of the continents. ---, Approximate positions in Central America, Eurasia and New Guinea of the southern boundaries of the North American and Eurasian plates and the northern boundaries of Indian and Australian plates which have moved rigidly since the Upper Cretaceous. The evolution of Middle America, the Mediterranean and the Alpide-Himalayan belt cannot be obtained in detail from oceanic magnetic lineations, and nothing is yet known about the evolution of South-east Asia. These regions have therefore been omitted. Elliptic projection centred on 0° latitude, 0° longitude, $x = R\phi \cos \lambda$, $y = R \sin \lambda$. 20° spacing between latitudes and longitudes.

specialized adaptations which often mimic the adaptations of the placental mammals that occur elsewhere. There are marsupial equivalents of cats, dogs, mice and moles, for example. They are assigned to three principal groups which occur only in Australia: the chiefly herbivorous phalangeroids (including kangaroos, wombats and opossums); the herbivorous perameloids (bandicoots); and the carnivorous dasyuroids (including the Tasmanian wolf, the banded anteater, and the marsupial mole). The seventy or so living American species are small unspecialized omnivores, insectivores and carnivores; many are arboreal and nocturnal. They are assigned to two principal groups: didelphoids (American opossums) and caenolestoids (opossum rats).

Many peculiar anatomical features suggest that all (and only) marsupials are descended from a single ancestral stock. This is partly confirmed by the cytology of living marsupials^{26,27}. This is important because it precludes explanations of their past and present distribution which presuppose that they evolved more than once in different parts of the world. Marsupial and placental mammals probably diverged at some time between the late Jurassic and mid-Cretaceous (between about -150 and about -100 Ma). They may both be derived from eupantotheres, small Jurassic mammals^{28,29}.

Before attempting to explain aspects of the evolution and dispersal of marsupials we shall outline what is known of their fossil record. The earliest known marsupials are didelphoids from the Cretaceous of North America. Slaughter³⁰ recognized a marsupial from the mid-Cretaceous (Albian, about -100 Ma) on the basis of molar teeth. Undoubted marsupials have been described from the upper Cretaceous

(Santonian, about -80 Ma)³¹. In the late Cretaceous didelphoid marsupials (which ranged from the mouse-like *Pedimys* to the dog-sized carnivore *Didelphodon*) underwent considerable differentiation in North America^{32,33}. At the end of the Cretaceous the North American marsupials were reduced in number and variety, probably because of competition with more specialized carnivorous placental mammals. Failure to find fossil marsupials in the late Cretaceous of Europe or Asia is not evidence that marsupials originated in North America. Only one late Cretaceous deposit of the Old World is known to have an extensive mammal fauna²⁸ and, because North America and Eurasia were effectively a single continent in the Cretaceous, it would be surprising if Cretaceous marsupials are not eventually discovered in the Old World.

Didelphoid marsupials have been found in the Palaeocene (about -60 Ma) of North America, Europe and South America. One of the North American late Cretaceous forms, *Alphadon*, had dental characteristics which suggest that it may have been ancestral to all the known Palaeocene didelphoids²⁸. Didelphoid marsupials became extinct in North America and Europe in the early Miocene. In the Palaeocene of South America appear the first known representatives of the caenolestoids and the carnivorous borhyaenoids³⁴.

Simpson²³ divided the Tertiary mammals of South America into three groups according to the presumed times at which their ancestors entered it from North America. First, there are mammals derived from late Cretaceous or earliest Palaeocene immigrants. These include the marsupials; a largely herbivorous group of placental mammals (litopterns, notoungulates and so on) derived from relatively unspecialized North Ameri-

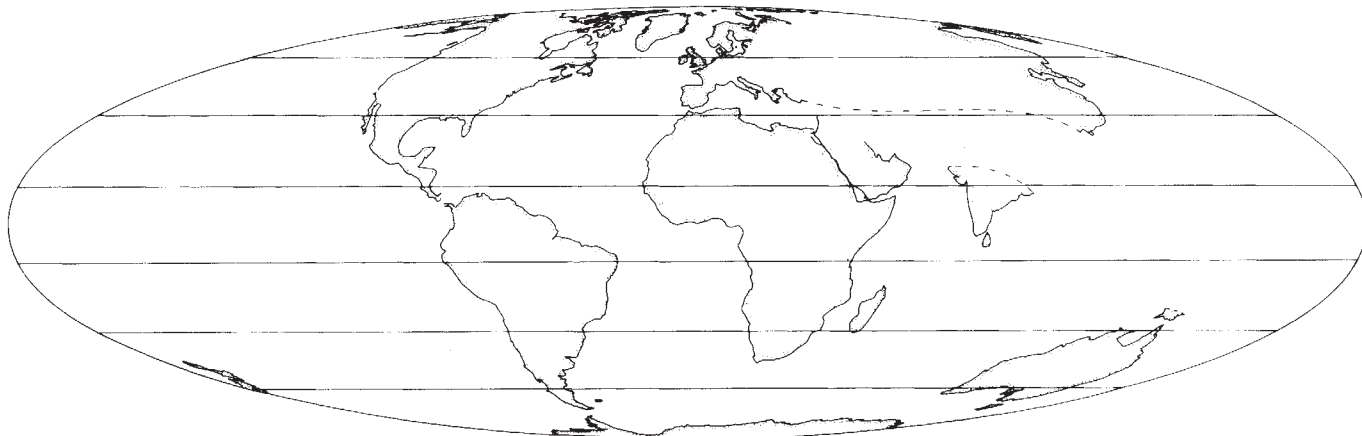


Fig. 2 Reconstruction (-35 Ma) obtained by removing all seafloor on which magnetic anomalies formed after -35 Ma were observed. South America is in contact with Antarctica, but Australia is not. Projection as in Fig. 1, but longitude lines omitted.

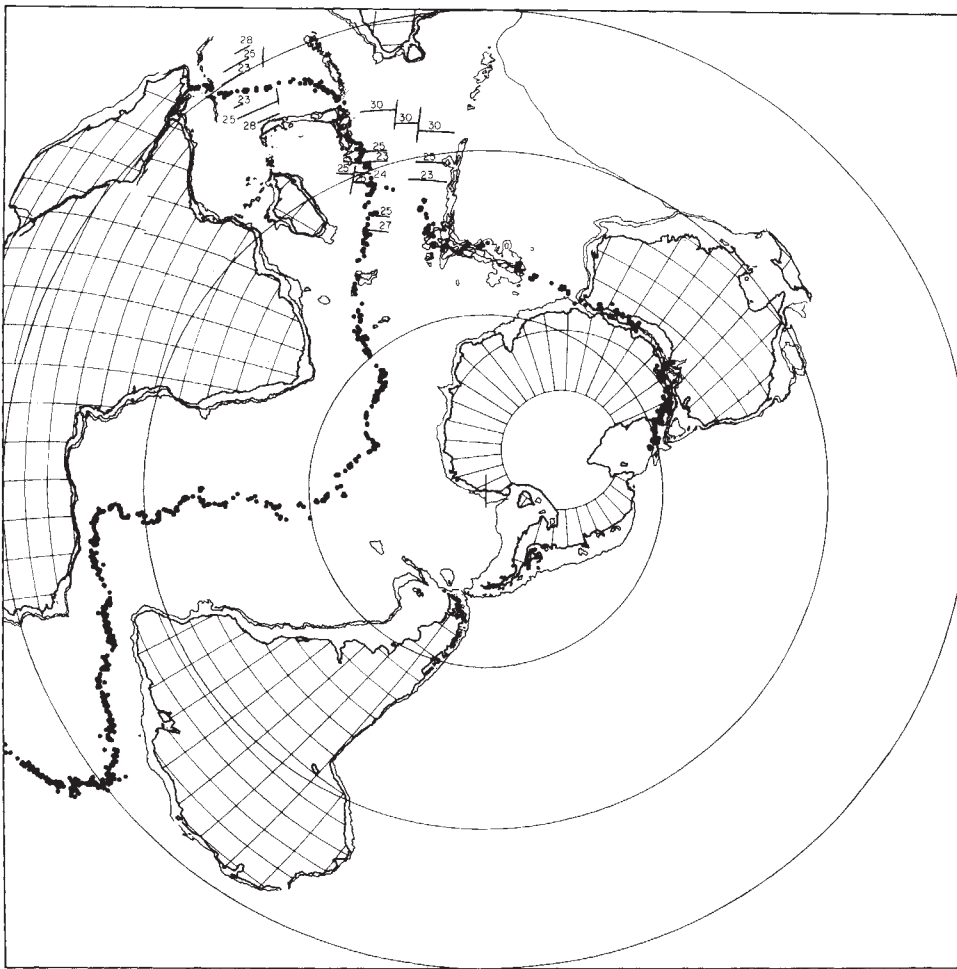


Fig. 3 Reconstruction (-45 Ma) obtained as in Fig. 2. ---, Active plate boundaries at this time; numbered straight lines, magnetic anomalies numbered according to Heirtzler *et al.*⁹. The 1 and 2 km bathymetric contours of the continental edges are shown. At this time Australia was connected to South America by Antarctica. Equal area projection centred on South Pole, with 30° spacing between latitudes (from McKenzie and Sclater¹²).

can placentals (ferungulates); and a group of "exotic" placentals (xenarthrans—including sloths, anteaters and armadillos) derived from another unspecialized North American stock (palaeonodonts). Second, there are New World monkeys and caviomorph rodents, probably derived from late Eocene or early Oligocene immigrants from North America. Third, there are the numerous and diverse specialized placental mammals which have invaded from North America since the late Miocene and which have largely displaced the older elements of the mammal fauna.

The hypothesis that the South American marsupials are derived from marsupial immigrants from North America is indirectly supported by evidence for the derivation of the other old elements of the South American fauna from North American immigrants. Borhyaenoids, the dominant carnivores of South America from the Palaeocene to the Pliocene, probably evolved from didelphoids in South America³⁵ but the place of origin of the caenolestoids is uncertain. Their Palaeocene representatives were already very distinct from didelphoids.

The hypothesis that all Australian marsupials are derived from immigrants from South America by way of Antarctica is based on more conclusive evidence. From the Albian/Aptian (about -106 Ma) when Africa separated from South America and Antarctica to -43 Ma (when Australia separated from Antarctica) this was the only available overland source for Australian marsupials. Cox's hypothesis³⁶ that marsupials reached Australia from Africa through Antarctica is ruled out by the early separation of Africa. Colonization of Antarctica by mammals during the late Cretaceous or early Tertiary is ecologically plausible. Antarctica was probably not glaciated during this period and the only known early Tertiary flora of Antarctica (Seymour Island, 64° S) indicates a cool temperate flora comparable with that of parts of New Zealand today^{37,38}.

The earliest known Australian marsupial *Wynyardia* is probably from the upper Oligocene (about -30 Ma)³⁹. Ride has shown that it has several primitive "didelphoid" features not found in later Australian marsupials¹⁸. By the Miocene Australian marsupials had undergone considerable differentiation, and fossils referable to three living families and one extinct family are known⁴⁰.

Serological studies of blood proteins suggest that in spite of their apparent diversity living Australian marsupials diverged from a common stock more recently than they diverged from either of the living South American groups^{19,27,41}. (Resemblances between the Australian phalangeroids and the South American caenolestoids, which have been thought to indicate an evolutionary link, could be the product of parallel evolution. Similarly the resemblances between the Australian dasyuroids and the extinct South American borhyaenoids are probably parallel carnivorous adaptations³⁵.) The distinctness of Australian and South American marsupials is indicated also by their spermatozoon morphology⁴² and development⁴³ and by the distinctness of their associated blood-sucking lice⁴⁴. These facts suggest that all the Australian marsupials originated from a single didelphoid stock which colonized Australia before its separation 43 Ma ago.

One outstanding question remains: why did marsupials alone reach Australia when both placentals and marsupials occurred in South America before -43 Ma (compare ref. 45). Any answer to this must be speculative. Adaptation and differentiation of marsupials and placentals in South America followed a very different course from that which occurred in North America²³. In North America, placental mammals gave rise both to the dominant specialized herbivores and to the dominant carnivores. Marsupials remained unspecialized and became extinct in the Miocene. In South America, placentals gave rise to highly specialized herbivores (litopterns and

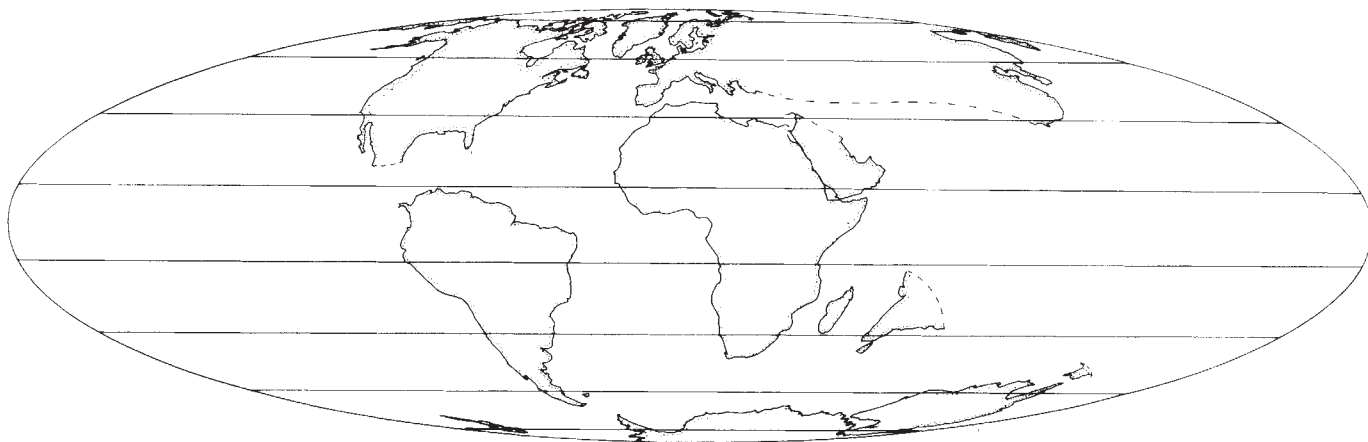


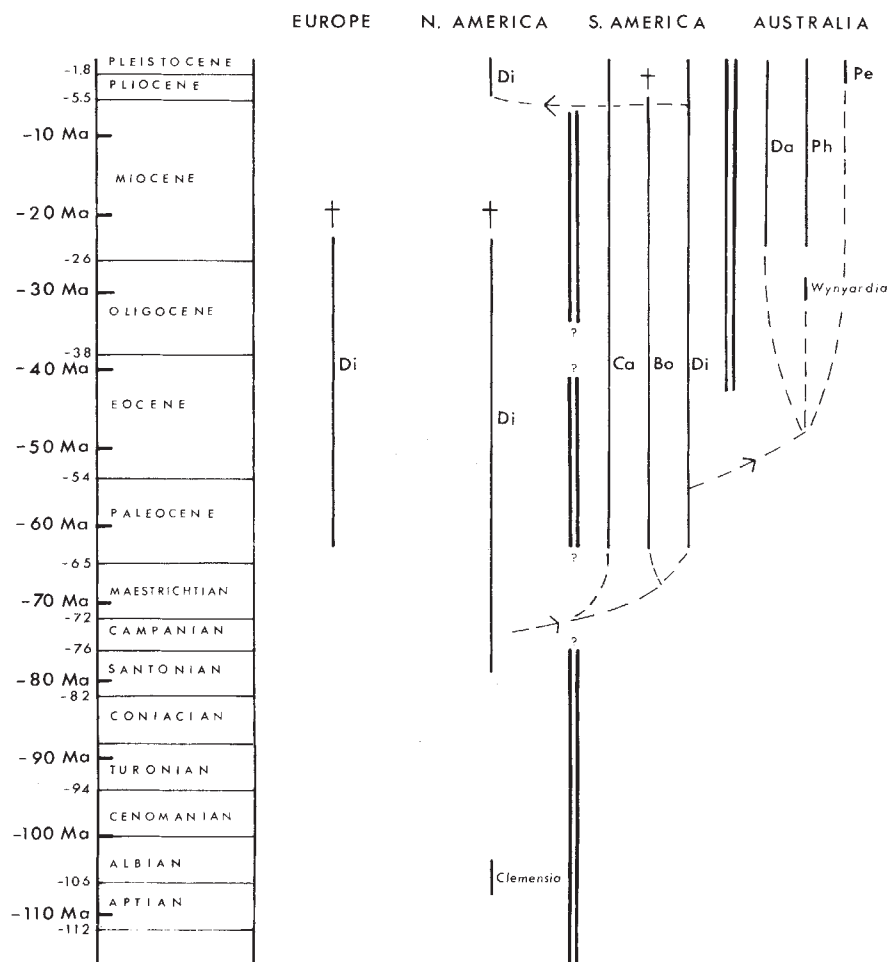
Fig. 4 Reconstruction (–75 Ma) obtained as in Fig. 2. India is still isolated and Africa does not provide a land connexion to Australia.

notoungulates) and to the specialized armadillos, anteaters and sloths (xenarthrans). Marsupials gave rise to the specialized carnivores (borhyaenoids), and relatively unspecialized omnivorous and insectivorous didelphoids persisted. It is reasonable to suppose that the unspecialized didelphoid marsupials were able to colonize Antarctica, and that the specialized placentals could not.

At the recent symposium "Organisms and Continents through Time" (Cambridge, December 1971), three alternatives to the above speculation were suggested. Cox⁴⁶ cited evidence that marsupials were present in South America in the Cretaceous when placentals may have been absent. He suggested that marsupials were at that time able to reach Antarctica, and that subsequently the route from South America to Australia *via* Antarctica became impassable to mammals either

through marine incursion between South America and Antarctica or through climatic change. Dr Pamela Robinson (personal communication) suggested that there may not have been a continuous land connexion between South America and Antarctica in the late Cretaceous and early Tertiary, and that marsupials alone may have won access to Antarctica by "island-hopping". Dr K. A. Joysey pointed out that, given the absence of mammalian records from Australia before the Oligocene, it remains possible that placentals and marsupials both reached Australia and that placentals subsequently became extinct. Each of these hypotheses is apparently consistent with the available evidence. The question may be resolved by further records of fossil marsupials and placentals, by further investigation of the plate tectonics of the Scotia Arc, or by discoveries about the climatic history of Antarctica.

Fig. 5 A reconstruction of marsupial evolution. Each solid line indicates persistence of one of the six principal groups of marsupials in a continent. A cross indicates extinction. Double lines indicate barriers to dispersal between continents. Dotted arrows indicate marsupial migrations which we have deduced. The six principal groups of marsupials are the superfamilies recognized by Simpson¹⁷. Di, Didelphoidea; Ca, Caenolestoidae; Bo, Borhyaenoidea; Da, Dasyuroidea; Ph, Phalangerioidea; Pe, Perameloidea. Groupings above the rank of superfamily have not been used because they are disputed^{18,19}. Our sources of information about fossil marsupial occurrences are ref. 20 and references cited there. The Tertiary stratigraphical scale is based on Berggren²¹. (The definition of the Oligocene/Miocene boundary differs from that adopted in ref. 20.) Dates of Cretaceous divisions are chiefly based on ref. 22. The date of separation of Australia rests on plate tectonic evidence. The dates of barriers between North and South America are inferred from biogeographical and palaeontological evidence^{23,24}. (Unfortunately the plate tectonics of the Caribbean region poses special difficulties. A change in sea-level of less than 100 m is sufficient to isolate South America.)



Guiding Principles

We conclude by discussing, with examples, some of the general principles which may guide biologists in combining knowledge of past continental arrangements with knowledge of past and present distributions of organisms in the study of evolution.

The history of the marsupials shows how breakup of a land mass, leading to isolation of organisms, provides opportunities for evolution in the absence of competitors which evolve elsewhere. It illustrates also how drifting continents may act as agents of dispersal. The history of the conifers provides another striking example of the action of drifting continents as agents of dispersal. Florin²⁴ showed that from the late Carboniferous (about -300 Ma) to the early Eocene (about -50 Ma) each of the conifer genera (with the exception of *Araucaria*) had either a "Gondwana" distribution (in one or more of South America, Antarctica, Africa, Madagascar, India, Australia, New Zealand) or a "Laurasian" distribution (in North America and/or Eurasia). During the Tertiary this neat division into two kinds of distribution has broken down. For example, fossil material of the "Gondwana" genus *Podocarpus* is known from the Mesozoic of South America, Australia, India, Antarctica and New Zealand. At present it occurs in all these regions except Antarctica, and has spread into Central America, Indonesia, South-east Asia and Japan. The present disjunct distributions of *Podocarpus* and the other Gondwana genera may be the product of the breakup of Gondwanaland; and the Tertiary spread of several of them into Indonesia and South-east Asia may result from the northward drift of Australia.

Both the conifers and the marsupials have relatively good fossil records and limited ability to cross water gaps. It is instructive to contrast them with the flowering plants which have a poor fossil record and which can disperse across oceans. Caution must be exercised in relating the evolution and distribution of such organisms to continental rearrangements. Disjunct distributions of families and genera of flowering plants which occur on remote oceanic islands cannot safely be attributed to separation of continents. The ancestors of all the flowering plants which occur as natives in the Hawaiian islands, for example, must have reached them by dispersal over long distances because they have been isolated by large ocean gaps since their volcanic origin since the Cretaceous. Further difficulties are caused by doubts about the validity of some of the families and genera which have striking disjunct distributions. The resemblances by which such families and genera are recognized may be the product of parallel evolution. Several facts about the present distributions of flowering plant families suggest, however, that much of the initial differentiation of flowering plants occurred in Gondwanaland before and during its breakup⁴⁷. First, it is striking that many flowering plant families with disjunct distributions (listed by Good⁴⁸) occur in two or more of South America, Africa, Madagascar and Australia. Many of these distributions may be the product of dispersal over long distances but this is a less likely explanation for the distribution of such families as the *Proteaceae*, several of whose genera have disjunct distributions. The Australasian and South American representatives of both the *Proteaceae* and the *Restionaceae* seem to be more closely related to each other than to the African representatives⁴⁹ and this may reflect the much earlier isolation of Africa from Gondwanaland. Second, several widespread northern flowering plant families are closely related to families which have more southern distributions and which have features that are believed to be more primitive. The northern family *Umbelliferae*, for example, is related to the more primitive tropical and southern family *Araliaceae*⁵⁰. Third, the apparently more primitive members of many cosmopolitan families and genera have southern distributions (see ref. 51).

The world's winds and ocean currents are known to be jointly determined by the disposition and surface topography of

the continents and the bathymetry of the oceans. It may eventually be possible to use knowledge of past continental arrangements to investigate past climatic changes and hence to explain aspects of the evolution of organisms. For example, the limited fossil record of the flowering plants suggests that they differentiated relatively rapidly during the early Cretaceous^{52,53} and it is tempting to relate this to relatively rapid climatic changes caused by the breakup of Gondwanaland.

- ¹ Hess, H. H., in *Petrological Studies: A Volume in Honor of A. F. Buddington* (edit. by Engel, E. A. J., et al.), 599 (Geological Society of America, New York, 1962).
- ² McKenzie, D. P., and Parker, R. L., *Nature*, **216**, 1276 (1967).
- ³ Morgan, W. J., *J. Geophys. Res.*, **73**, 1959 (1968).
- ⁴ Barazangi, M., and Dorman, J., *Bull. Seismol. Soc. Amer.*, **59**, 369 (1969).
- ⁵ Vine, F. J., and Matthews, D. H., *Nature*, **199**, 947 (1963).
- ⁶ Wilson, J. T., *Nature*, **207**, 343 (1965).
- ⁷ Pitman, W. C., Herron, E. M., and Heirtzler, J. R., *J. Geophys. Res.*, **73**, 2069 (1968).
- ⁸ Dickson, G. O., Pitman, W. C., and Heirtzler, J. R., *J. Geophys. Res.*, **73**, 2087 (1968).
- ⁹ Heirtzler, J. R., Dickson, G. O., Herron, E. M., Pitman, W. C., and Le Pichon, S., *J. Geophys. Res.*, **73**, 2119 (1968).
- ¹⁰ Bullard, E. C., Everett, J. E., and Smith, A. G., *Phil. Trans. Roy. Soc. A*, **258**, 41 (1965).
- ¹¹ Le Pichon, X., *J. Geophys. Res.*, **73**, 3661 (1968).
- ¹² McKenzie, D. P., and Sclater, J. G., *Geophys. J. Roy. Astron. Soc.* (in the press).
- ¹³ Pitman, W. C., Talwani, M., and Heirtzler, J. R., *Earth Planet. Sci. Lett.*, **11**, 195 (1971).
- ¹⁴ Williams, C. A., and McKenzie, D. P., *Nature*, **232**, 168 (1971).
- ¹⁵ Pitman, W. C., and Talwani, M., *J. Geophys. Res.* (in the press).
- ¹⁶ Simpson, G. G., *Evolution*, **15**, 431 (1961).
- ¹⁷ Simpson, G. G., *Fossilium Catalogus, I: Animalia*, Pars 47 (Junk, Berlin, 1930).
- ¹⁸ Ride, W. D. L., *J. Roy. Soc. W. Austral.*, **47**, 97 (1964).
- ¹⁹ Kirsch, J. A. W., *Nature*, **217**, 418 (1968).
- ²⁰ *The Fossil Record* (edit. by Harland, W. B., et al.) (Geological Society, London, 1967).
- ²¹ Berggren, W. A., *Micropalaeontology*, **15**, 351 (1969).
- ²² Harland, W. B., Smith, A. G., and Wilcock, B., *Quart. J. Geol. Soc. Lond.*, **120**, Suppl. (1964).
- ²³ Simpson, G. G., *Amer. Scient.*, **38**, 361 (1950).
- ²⁴ Florin, R., *Acta Hort. Berg.*, **20**, 1 (1963).
- ²⁵ Walker, E. P., *Mammals of the World*, **1** (Johns Hopkins, Baltimore, 1964).
- ²⁶ Sharman, G. B., *Austral. J. Zool.*, **9**, 38 (1961).
- ²⁷ Hayman, D. L., Kirsch, J. A. W., Martin, P. G., and Waller, P. F., *Nature*, **231**, 194 (1971).
- ²⁸ Clemens, W. A., *Evolution*, **22**, 1 (1968).
- ²⁹ Hopson, J. A., and Crompton, A. W., in *Evolutionary Biology*, (edit. by Hecht, M. K., and Steere, W. C.), **3**, 15 (North-Holland, Amsterdam, 1969).
- ³⁰ Slaughter, B. H., *Science*, **162**, 245 (1968).
- ³¹ Russell, L. S., *Bull. Nat. Mus. Canad.*, **126**, 110 (1952).
- ³² Clemens, W. A., *Univ. Calif. Pub. Geol. Sci.*, **48**, 1 (1966).
- ³³ Lillegraven, J. A., *Univ. Kansas Palaeont. Cont.*, **50**, 1 (1969).
- ³⁴ Paula Couto, C. de, *Amer. Mus. Novit.*, **1959**, 11 (1952).
- ³⁵ Simpson, G. G., *Amer. Mus. Novit.*, **1118**, 1 (1941).
- ³⁶ Cox, C. B., *Nature*, **226**, 767 (1970).
- ³⁷ Dusen, P., *Wissenschaftl. Ergebn. Schwed. Südpolarexped.*, **3**, 3 (Stockholm, 1908).
- ³⁸ Cranwell, L. M., *Nature*, **184**, 1782 (1959).
- ³⁹ Gill, E. D., *Mem. Nat. Mus. Vict.*, **21**, 135 (1957).
- ⁴⁰ Stirton, R. A., Tedford, R. H., and Miller, A. H., *Rec. S. Austral. Mus.*, **14**, 19 (1961).
- ⁴¹ Weymss, C. T., *Zoologica*, **38**, 173 (1952).
- ⁴² Hughes, R. L., *Austral. J. Zool.*, **13**, 533 (1965).
- ⁴³ Biggers, J. D., and De Lamater, E. D., *Nature*, **208**, 402 (1965).
- ⁴⁴ Vanzolini, P. E., and Guimarães, L. R., *Evolution*, **9**, 345 (1955).
- ⁴⁵ Ride, W. D. L., in *Evolution of Living Organisms* (edit. by Leeper, C. W.), 281 (Melbourne University Press, 1962).
- ⁴⁶ Cox, C. B., paper read at Symposium on Organisms and Continents through Time (Cambridge, December 1971).
- ⁴⁷ Hawkes, J. G., and Smith, P., *Nature*, **207**, 48 (1965).
- ⁴⁸ Good, R., *The Geography of the Flowering Plants*, third ed. (Longmans, Green, London, 1964).
- ⁴⁹ Cutler, D. F., *Conference on Taxonomy and Phytogeography of the Flowering Plants in Relation to Evolution* (Manchester, September 1971).
- ⁵⁰ Camp, W. H., *Ecol. Monogr.*, **17**, 160 (1947).
- ⁵¹ Croizat, L., *Bull. Torrey Bot. Club*, **47** (1947).
- ⁵² Scott, R. A., Barghoorn, E. S., and Leopold, E. B., *Amer. J. Sci.*, **258A**, 284 (1960).
- ⁵³ Muller, J., *Biol. Rev.*, **45**, 417 (1970).

Cosmic Ray Isotropy and the Origin Problem

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A study of the cosmic ray muon intensity underground in London has revealed small sidereal variations which can be explained in terms of solar motion.

No firm conclusion has been reached as to how much of the cosmic ray flux at the Earth arises from sources inside our own galaxy and how much is of extra-galactic origin. Perhaps the most widely held view is that most of the cosmic rays, with the possible exception of those of extremely high energy ($\geq 10^{18}$ eV), are generated within our own galaxy either in supernovae explosions or subsequently during the braking of the rotation of the pulsar relics of such explosions¹. Following their acceleration, the cosmic rays are supposedly contained within the Galaxy for periods of 10^6 to 10^7 yr and during this time are rendered isotropic by the galactic magnetic field which is believed to have an average strength of a few micro-gauss.

In these circumstances the degree of isotropy of the particle flux at the Earth should depend on the source distribution and on the escape time from the Galaxy. In general, the more isotropic the flux the greater must be the escape² time. An independent estimate of the cosmic ray lifetime in the Galaxy can be obtained from the abundance of the light elements which are produced by fragmentation of higher $-Z$ nuclei and comparison of this lifetime with that required by the measured degree of isotropy of the radiation provides, therefore, a test of the internal consistency of the galactic origin hypothesis.

We have recently reported the results of a search for a sidereal daily variation in the muon intensity at 60 m.w.e. depth which relates to the isotropy of cosmic rays with magnetic rigidity $R \gtrsim 10^{11}$ V (ref. 3). The interpretation of these measurements depends critically on the extent to which the particle trajectories are affected by the interplanetary magnetic field. At low energies, where the Larmor radii in the interplanetary field are small compared with 1 AU, any isotropy existing in interstellar space is completely washed out by the local field and it has been suggested that the low value for the anisotropy reported by us⁴ might be explained in this way. At higher energies where the Larmor radius is appreciably greater than 1 AU the interplanetary scattering can certainly be ignored but in this case the fraction of the total cosmic ray energy flux to which the measurements apply may be quite small because of the steepness of the intensity/rigidity spectrum. Primary cosmic rays with rigidity greater than 10^{11} V carry $\sim 10\%$ of the total energy flux and measurements in this part of the spectrum can therefore be regarded as reasonably representative provided they are not invalidated by scattering in the interplanetary field. We will show that this scattering does not seriously obscure a genuine galactic anisotropy at these energies.

A preliminary semi-quantitative treatment of the motion of cosmic rays in the interplanetary magnetic field was given in ref. 4 and this has now been extended to permit a refinement of the earlier analysis and interpretation of the measurements.

The implications for theories of the origin of cosmic rays are discussed below.

Propagation of Cosmic Rays

The average properties of the interplanetary magnetic field in that region of space lying between the orbits of Venus and Mars and close to the ecliptic plane are well known. On the average, the field direction seems to be reasonably consistent with the spiral configuration to be expected on the Parker solar wind model. Strictly speaking, the spiral form corresponds to an idealized situation in which the wind speed is independent of time and of solar longitude, and at any one time it is likely to be appreciably disturbed from this idealized form because of variability in the wind speed, both in time and in solar longitude. A turbulent field is less effective in deviating cosmic ray particles than one which is uniform, and adoption of the smooth field model is therefore expected to give an upper limit to the effect of the field on cosmic ray trajectories. Measurements over the past decade give a most probable value of $|B| \sim 5 \times 10^{-5}$ Oe at 1 AU and have shown that in the plane of the ecliptic the field generally has a large scale sector structure with alternate negative and positive sectors having field directed respectively into and out of the Sun. The number of sectors has varied from two in 1962 to four in 1964 to two in 1970. We have chosen to represent the field as having four sectors but emphasize that the results are not appreciably changed if a two sector structure is adopted instead. This field has an average value $|B| = 5 \times 10^{-5}$ Oe at 1 AU with a spiral form making an angle of $\sim 45^\circ$ with the Earth-Sun line. This angle corresponds to a solar wind speed of 370 km s^{-1} . We have made the further assumption that this velocity is independent of time and heliographic latitude and longitude. We also assume that the root field at the base of the corona is uniform and constant in time. Thus the field components are defined by:

$$\begin{aligned} B_r &= \frac{3.5 \times 10^{-5}}{r^2} \\ B_\theta &= 0 \\ B_\phi &= \frac{3.5 \times 10^{-5}}{r} \cos \theta \end{aligned}$$

Where r is the distance from the Sun measured in AU, θ and ϕ are heliographic latitude and longitude respectively.

Using this field model the equations of motion for negatively charged particles emitted from the Earth in various directions have been integrated and the asymptotic direction of the trajectories at infinity determined. In practice the integration was stopped at $r = 50$ AU because the deflexions at greater distances are negligible. This is an equivalent but more convenient procedure than integrating the equations for positive particles coming in from infinity towards the Earth.

The starting conditions have been varied for direction, position of the Earth relative to the sector structure and particle energy so as to produce a representative selection covering the Earth's motion during the year and the relevant part of the primary energy spectrum. Asymptotic directions at 50 AU were determined at two hourly intervals throughout the sidereal day for primaries with rigidity 100, 150, 200, 300, 500 and

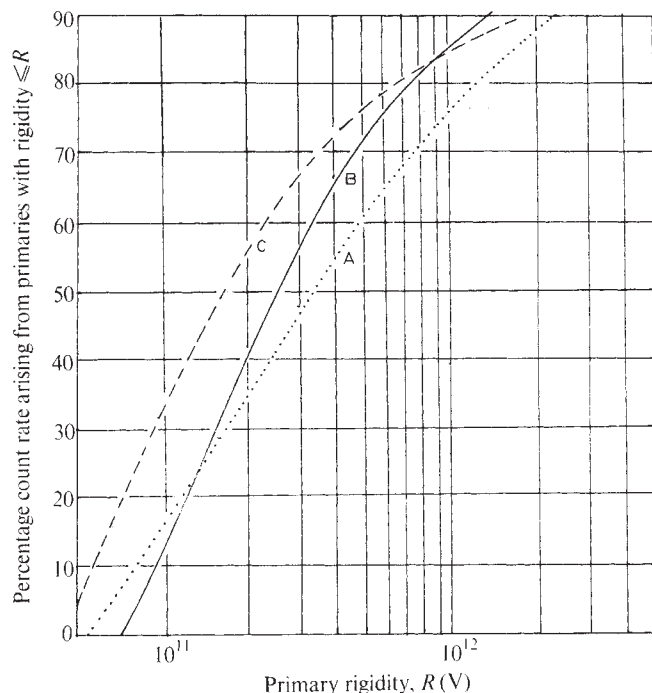


Fig. 1 Response functions for a muon detector at a depth of 60 m.w.e. Curve A (Peacock), curve B (Wolfendale), curve C (Ahluwalia and Ericksen).

9,000 GV. In each case the computations were carried out for the solstitial and equinoctial positions of the Earth taking it first to be at the mid-point of a negative sector and then at the mid-point of a positive sector. In all, eight asymptotic directions were determined for each even hour of the sidereal day.

The asymptotic directions for low rigidity primaries depend critically both on the starting conditions and on the rigidity with the result that the interplanetary magnetic field can be expected to smear out virtually all trace of a genuine cosmic ray anisotropy at magnetic rigidities less than ~ 150 GV. The amount of scatter diminishes with increasing rigidity becoming relatively unimportant above ~ 300 GV. This behaviour is illustrated in Fig. 4. In these diagrams α is the angle between the asymptotic cosmic ray directions and the solar apex at RA 18 h, $\delta = 34^\circ$ N. For each value of rigidity $\cos \alpha$ is plotted at two hourly intervals throughout the sidereal day for the eight starting conditions given above. The decrease in scatter with increase in magnetic rigidity is clearly seen. All primary cosmic rays above some minimum threshold rigidity contribute to some extent to the counting rate of an underground recorder and in order to compute the effective scan across the celestial sphere that is made by such a recorder it is necessary to make use of the underground response function which specifies the fraction of the counting rate arising from each rigidity interval in the primary spectrum.

Underground Response Function

Peacock⁵ derived a response function for a depth of 60 m.w.e. by using the Trilling formula to represent the pion multiplicity as a function of energy and a primary integral energy spectrum of the form $E_p^{-1.67}$. At high energies the Trilling formula asymptotically approaches a pion multiplicity which is proportional to $E_p^{1/2}$. Curve A in Fig. 1 has been derived using the same primary spectrum as Peacock but under the assumption that the Trilling formula applies at all energies whereas Peacock neglected all secondary production at $E_p > 2 \times 10^{12}$ eV.

Curve B has been derived by Wolfendale (personal communication) and is based on an integral energy spectrum of the form $N(>E_p) \sim E_p^{-1.5}$, a pion multiplicity given by $M = 0.8 E_p^{1/2}$ (where E_p is in GeV) and the CKP model of high energy inter-

actions. Curve C is a response function derived by Ahluwalia *et al.*⁶. In each case the curves have been adjusted to take into account the primaries with $Z/A = \frac{1}{2}$ so that the response can be plotted as a function of magnetic rigidity rather than energy since it is, of course, the magnetic rigidity which fixes the particle trajectory in the interplanetary field.

Curves B and C have been used to calculate the directions on the celestial sphere scanned by the underground detector. These are plotted in Fig. 2. Also shown in this diagram is the corresponding scan for the infinite rigidity case. Curve C places much greater weight on the contribution to the counting rate by low rigidity primaries and we believe that curve B more correctly represents the real situation. The conclusions reached would not be materially altered if we were to adopt curve C instead.

Cosmic Ray Sidereal Daily Variation

The detectors used for monitoring the muon intensity were installed in an underground laboratory at the Holborn station of the London Transport Executive. They consisted of two-fold coincidence telescopes using one inch thick slabs of plastic scintillator arranged in semi-cubical geometry and viewed by 5 inch photo multipliers. The telescopes pointed vertically and each had a sensitive area of 1.44 m^2 giving a counting rate of 25,000/h. Two such telescopes were in operation from November 1960 to October 1962, six from April 1963 to July 1964 and two from October 1964 to September 1969.

In an experiment of this kind it is, of course, desirable to have an estimate of the magnitude of the variation to be

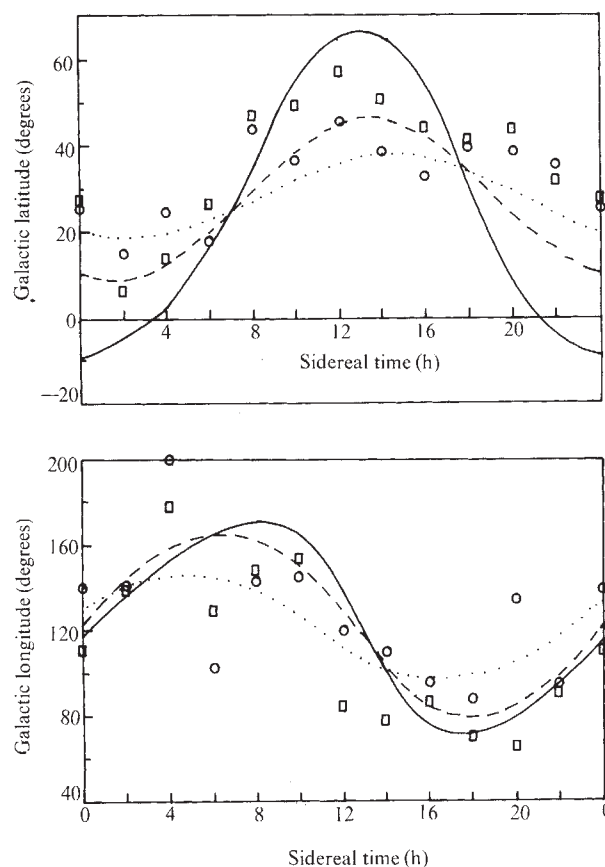


Fig. 2 Scans in galactic latitude and longitude as a function of sidereal time for a vertical telescope at London. $\square$, Mean values using response function B to weight individual calculated values. The dashed line is the best fitting sine curve through these points. $\circ$, Results weighted according to Ahluwalia's response function; the dotted line is the best fitting sine curve to these points. The solid line is the scan produced by considering only infinite energy particles.

expected. At the time when this experiment was planned the only well established result was that the amplitude of the sidereal daily variation at 60 m.w.e. depth was less than 0.1%. In principle, there is no reason why the galactic magnetic field together with a suitably uniform distribution of sources in the Galaxy should not make the cosmic rays isotropic to a higher degree than this. Nevertheless, no matter how effective the field is in making the radiation isotropic, a limit is set ultimately by the frame of reference in which the observations are made. For instance, one may imagine the radiation to be perfectly isotropic in the frame corresponding to the local rotational velocity of the interstellar material in which the magnetic field is fixed. Relative to this frame the solar system has a velocity of $\sim 20 \text{ km s}^{-1}$ in the direction of the constellation Hercules and under these circumstances an observer on the Earth should see an anisotropy of two or three parts in 10^4 corresponding to this motion. The detector size for the Holborn installation and the time scale of the experiment were chosen in order to establish whether such an anisotropy due to solar motion really exists.

Fig. 3 shows the average bi-hourly departures from the mean plotted in sidereal time for the whole period of observation. The error bars are standard deviations appropriate to the bi-hourly counting rate. The data have been corrected for variations in barometric pressure.

If the radiation is assumed to be completely isotropic in the frame of galactic rotation, that is, the cosmic rays co-rotate with the Galaxy, the angular distribution seen by an observer in the solar system is given by:

$$I = I_0 \left[1 + (2 + \gamma) \frac{v}{c} \cos \alpha \right]$$

where I_0 is the mean intensity, γ is the exponent of the differential energy spectrum, v is the velocity of the solar system relative to the rotating frame and α is the angle between the direction of observation and the solar apex.

Fig. 4 shows $\cos \alpha$ plotted in sidereal time for six different values of primary magnetic rigidity and for the eight representative starting conditions already given. The dotted curves are sine waves of best fit and the increase in amplitude of these fitted waves with increasing magnetic rigidity reflects the progressive decrease in scattering by the interplanetary magnetic field at the higher rigidities. The mean values of $\cos \alpha$ together with response function B of Fig. 1 and the expression given above for the intensity have been used with $\gamma=2.5$ to compute the sidereal daily variation to be expected for the underground recorder due to solar motion and this is represented by the curve shown in Fig. 3. It is apparent that there is good agreement between the observed departures from the mean intensity and this curve. Fig. 5 shows the residual departures after subtracting the computed curve as a correction and it is clear that these residuals do not show any systematic variation over the sidereal day which is greater than the statistical noise. Deflexion of the cosmic ray primaries in the geomagnetic field has the effect of displacing the computed curve towards earlier sidereal time by about half an hour but in view of other much larger uncertainties no correction has been applied for this effect.

Fig. 6 shows the first and second harmonics of the residual bi-hourly departures of Fig. 5 plotted on a sidereal time dial. The circles correspond to 99% confidence limits and have been computed from the scatter of the harmonic coefficients determined from a large number of sub-groups. The standard deviation computed in this way is about 1.4 times that which would be expected on the basis of the counting statistics alone.

Significance of the High Degree of Isotropy

The remarkably high degree of isotropy of cosmic rays in the energy range 10^{11} – 10^{12} eV which has been established in the series of measurements described above can be accounted

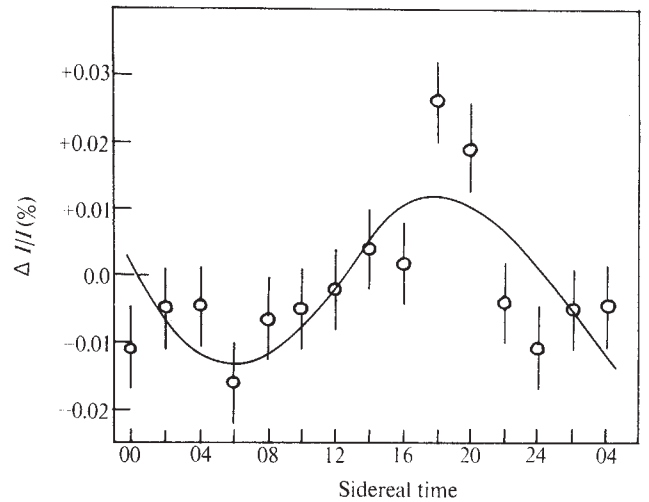


Fig. 3 ○, Average bi-hourly departures from the mean of the muon intensity at 60 m.w.e. plotted as a function of sidereal time. The curve is the variation to be expected on account of the motion of the solar system relative to the local galactic rotation frame of reference.

for quite readily if these particles have very much the same density in intergalactic space as in the Galaxy. In these circumstances the presence of a galactic magnetic field ensures that the cosmic rays observed at the Earth co-rotate with the Galaxy so that there is no Compton-Getting effect which otherwise would be an order of magnitude greater than the solar motion effect discussed above. If, however, cosmic rays in this energy band belong to a predominantly galactic population, the observed degree of isotropy has to be explained in terms of diffusion and containment in the galactic field and an appropriate source distribution.

In an isotropically diffusing region containing a uniform source distribution the anisotropy of cosmic rays defined by:

$$\delta = \frac{I_{\max} - I_{\min}}{\frac{1}{2}(I_{\max} + I_{\min})}$$

is given by:

$$\delta = k \frac{L}{c\tau} \quad (1)$$

where L is the smallest dimension of the diffusing region, τ is the lifetime of the cosmic rays against escape, c is the particle velocity and k is a numerical constant which is close to unity provided the point of observation is not too close to either the boundary of the diffusing region or a position of symmetry.

For isotropic diffusion with a transport free path, λ , the mean square distance reached after N steps is given by:

$$d^2 = 2N\lambda^2 \quad (2)$$

When d approaches the dimension of the scattering region, L , the particles will escape and we may therefore write:

$$c\tau = N\lambda \quad (3)$$

Combining (1), (2) and (3) gives:

$$d^2 = 2c\tau\lambda \quad (4)$$

and

$$\delta^2 = \frac{2\lambda}{c\tau} \quad (5)$$

The observed abundance of the light nuclei, Li, Be and B, in the primary cosmic ray beam sets an upper limit to τ of about 10^7 years for containment in the disk of the galaxy and if we take $\delta \leq 10^{-4}$ (4) gives $\lambda \leq 0.1$ l.y. A value of the diffusion mean free path as low as this does not correspond to the

observed properties of the interstellar magnetic field and must therefore be considered implausible.

Lingenfelter *et al.*⁷ have shown that this difficulty can be avoided if the cosmic rays are assumed to diffuse in a stochastic field of the kind envisaged by Jokipii and Parker² where the field lines themselves describe a random walk and the cosmic rays are subject to one-dimensional diffusion along these field lines. Because of the random motion of the field lines associated with the turbulent motion of the interstellar plasma all field lines intersect the surface of the galactic disk every few thousand light years. In these circumstances and assuming a uniform source distribution Lingenfelter *et al.* show that the escape time from the galactic disk by diffusion is given by:

$$\tau = \frac{L^2}{\lambda_1 \lambda_2^2 c}$$

where L is the distance measured in the plane of the Galaxy that the particles have to travel in order to escape from the disk, λ_1 is the scattering mean free path for one-dimensional diffusion along a field line and λ_2 is the characteristic step length for the random walk of the field lines themselves.

Taking $L = 1,500$ l.y. and $\lambda_1 = \lambda_2 \approx 100$ l.y. gives $\tau \approx 5 \times 10^6$ yr so that the model leads to a cosmic ray lifetime in the disk of the right order with plausible values of λ_1 and λ_2 . In this model convection of the field lines due to turbulence of the interstellar plasma can also contribute to the escape of cosmic rays from the disk. Lingenfelter *et al.*⁴ estimate the escape time for convection alone to be $\sim 10^7$ yr so that both processes are likely to contribute significantly to the loss of cosmic rays. Working on the assumption that cosmic rays are produced by supernova and then propagate throughout the Galaxy by

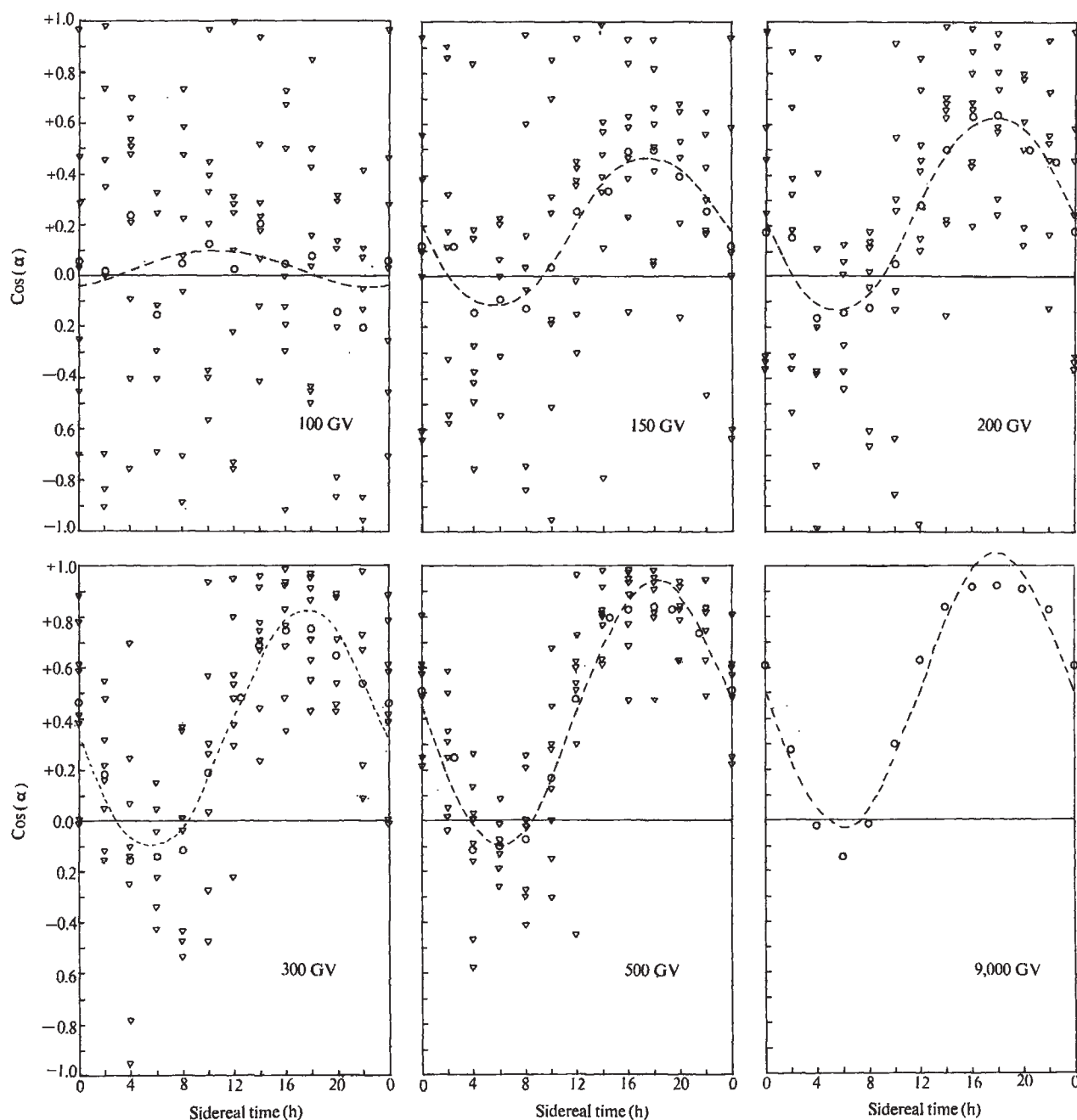


Fig. 4 The angular scan of the muon telescope relative to the direction of solar apex for primaries with magnetic rigidity 100, 150, 200, 300, 500 and 9,000 GV. α is the angle between the solar apex and the effective viewing direction calculated for the eight starting conditions given in the text. The mean values are indicated by ∇ . The curve drawn through the points is the sine wave of best fit.

multiple diffusion, Ramaty *et al.* have calculated the anisotropy to be expected in these conditions. The values obtained, which are based on a random distribution of supernova with a frequency of one per 100 yr in the Galaxy, are:

$$\delta = \left[1.4^{+3.1}_{-0.9} \right] \times 10^{-4} \text{ for } = 5 \times 10^6 \text{ yr}$$

and
$$= \left[0.75^{+1.9}_{-0.5} \right] \times 10^{-4} \text{ for } = 15 \times 10^6 \text{ yr}$$

In each case it was assumed that $\lambda_1 = \lambda_2 = 30$ pc. The relative large fluctuations on the mean value of δ , which correspond to 1σ limits, reflect the statistical uncertainty in the positions and ages of the closest cosmic ray sources.

It is apparent that the upper limit to the value of δ measured in the present experiment lies within the limits estimated by Ramaty *et al.* and there is clearly no inconsistency between their model and the experimental result reported here. This situation would, however, be changed if there were a nearby source of cosmic rays contributing a sizable fraction of the observed intensity. In this connexion it is interesting to note that Lingenfelter⁷ concluded that if pulsars were the source of cosmic rays something in the region of 10 to 20% of the currently observed flux could be due to PSR 1929+10, whose age he estimated to be $\sim 6 \times 10^4$ yr. Such a source would produce an anisotropy with δ in the region of 4 to 10×10^{-4} and should be observable in the present experiment. It now seems, however, that the age of PSR 1929+10 may be as great as 3×10^6 yr (ref. 8) in which case the contributions from this source would constitute a very small fraction of the current flux of cosmic rays and the anisotropy would be correspondingly small—well below the level of detectability in this experiment. When a reasonably complete catalogue of pulsar ages and distances becomes available it will be possible to compute the anisotropy to be expected if pulsars are indeed the source of cosmic radiation. In principle, experiments of the kind described here could then permit an experimental test of the pulsar origin hypothesis but it will not be easy to realize this in practice because of the difficulty in separating the anisotropy produced by the source distribution from that produced by the solar motion.

Meanwhile, the upper limit to the cosmic ray anisotropy of $\delta \leq 1.5 \times 10^{-4}$, after correction for solar motion, appears to be compatible with a galactic origin coupled with the compound diffusion model of Lingenfelter *et al.* in the energy range 10^{11} to 10^{12} eV. It would, of course, also be compatible with a universal distribution of these particles throughout the metagalaxy. The most cogent argument against a universal cosmic ray population at these energies rests on the improbably large power of the sources required to sustain a universal cosmic ray energy density of ~ 0.1 eV cm⁻³ but the force of this argument

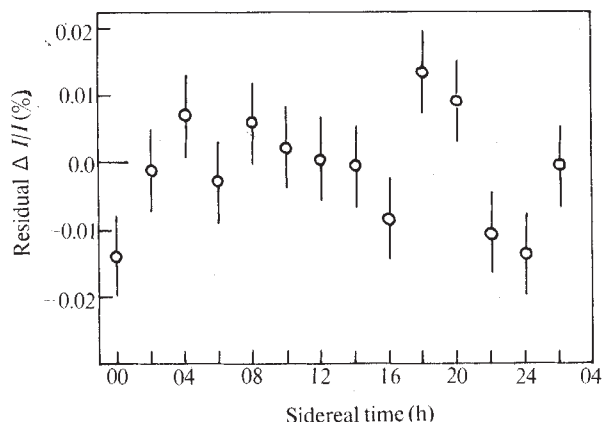


Fig. 5 The bi-hourly residual departures of the muon intensity at 60 m.w.e. after correction for solar motion.

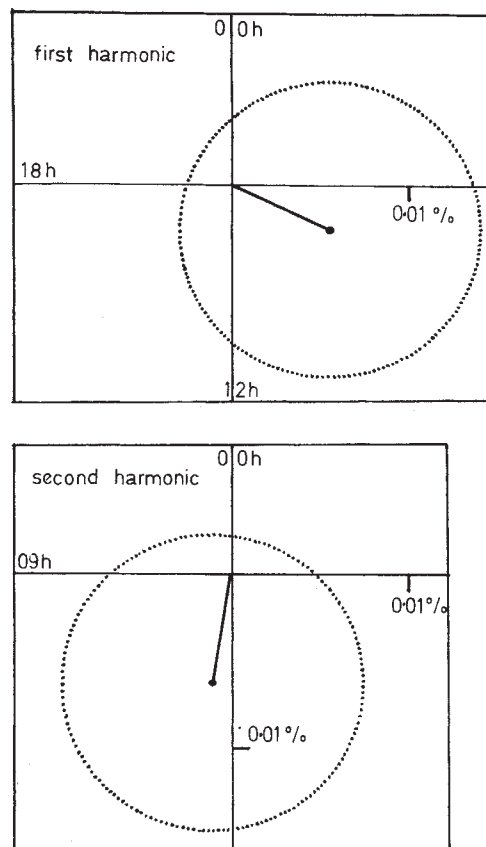


Fig. 6 Sidereal time dials showing the first and second harmonics of the residual bi-hourly departures of Fig. 5 after correcting for solar motion.

has diminished in recent years as has recently been pointed out by Setti and Woltzer⁹ and the possibly universal distribution of these particles has to be taken seriously.

Summary

A detailed study of the trajectories of cosmic rays of magnetic rigidity $\geq 10^{11}$ V in the interplanetary magnetic field has shown that the small but statistically significant sidereal variation present in the cosmic ray muon intensity at a depth of 60 m.w.e. in London³ can be interpreted in terms of the known motion of the solar system relative to the local galactic rotation frame.

When the effect of this solar motion is removed there is no statistically significant residual variation over the sidereal day. The residual amplitude is $(0.006 \pm 0.008)\%$ at the 2σ confidence level. This result is compatible with a galactic origin for the particles in question, provided that they can be assumed to propagate through the Galaxy by compound diffusion as proposed by Lingenfelter *et al.*⁴. It is also compatible with any other galactic origin model which requires a cosmic ray streaming velocity ≤ 30 km s⁻¹ or with a universal distribution of these particles where the metagalactic energy density is not greatly different from that at the Earth.

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- ¹ Gold, T., *Nature*, **221**, 25 (1969).
- ² Jokipii, J. R., and Parker, E. N., *Astrophys. J.*, **155**, 799 (1969).
- ³ Elliot, H., Thambyahpillai, T., and Peacock, D. S., *Acta Phys. Hung.*, **29**, Suppl. 1, 491 (1970).
- ⁴ Lingenfelter, R. E., Ramaty, R., and Fisk, L. A., *Astrophys. Lett.*, **8**, 93 (1971).
- ⁵ Peacock, D. S., *Acta Phys. Hung.* (in the press).
- ⁶ Ahluwalia, H. S., and Ericksen, J. H., *J. Geophys. Res.*, **76** (in the press).
- ⁷ Lingenfelter, R. E., *Nature*, **224**, 1182 (1969).
- ⁸ Hunt, G. C., *Mon. Not. Roy. Astron. Soc.*, **153**, 119 (1971).
- ⁹ Setti, G., and Woltzer, L., *Nature*, **231**, 57 (1971).

Ribosomal RNA Genes and Plant Development

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The number of copies of the ribosomal RNA genes varies considerably between the different plant species studied. It does seem, however, that there is no gross amplification or deletion of these genes during the development of a particular plant.

AMPLIFICATION of the ribosomal-RNA (rRNA) genes occurs during oocyte development in amphibia¹ and possibly in the eggs of other animals². Plants contain a large number of copies of rRNA genes³, but the possible amplification of these genes during plant development is little known. Since plants undergo many dramatic changes in metabolism, from dormant to actively growing states, and these gross metabolic changes invariably involve large changes in the rate of accumulation of rRNA, it is interesting to determine the number of rRNA genes in a range of species, and their quantitative variation with different phases of development.

Number of rRNA Genes

We studied this problem by molecular hybridization of rRNA to DNA⁴. DNA was prepared from total plant tissue or from nuclear preparations⁵, and purification on a preparative CsCl gradient was necessary for reproducible hybridization. Radioactive RNA was prepared from long-labelled plants or plant tissue⁶, and fractionated by polyacrylamide gel electrophoresis⁷. Slices (0.5 mm) containing the two high molecular weight rRNAs (1.3×10^6 and 0.7×10^6) were cut from the frozen gel and the RNA eluted with $6 \times \text{SSC}$ (SSC is 0.15 M NaCl, 0.015 M Na citrate, pH 7.2) at 50°C for 2 h. The RNA collected by centrifugation at 120,000g for 16 h was quantitatively recovered with essentially no degradation.

The rRNA (2 to 5 $\mu\text{g}/\text{ml}$) was hybridized to the DNA (15 to 25 $\mu\text{g}/\text{filter}$) at 70°C in $6 \times \text{SSC}$. The rate of hybridization was very fast in these conditions, with 50% reaction occurring after 3 to 5 min (RNA at 5 $\mu\text{g}/\text{ml}$). Saturation levels were determined by incubations for 2 h. The DNA content of the filters was determined by acid hydrolysis after

counting⁸. The 0.7×10^6 molecular weight rRNA hybridizes to the 1.3×10^6 molecular weight DNA component in addition to the 0.7×10^6 site, whereas hybridization of 1.3×10^6 molecular weight rRNA to the 0.7×10^6 molecular weight gene is very much less (N. S. Scott and J. I., unpublished work). Consequently, saturation levels of the large rRNA have been used for quantitative determinations.

Table 1 Number of rRNA Genes in Different Plants

Plant	% DNA hybridized	DNA 10^{-12} g/ telophase nucleus	Genes/ telophase nucleus	Ploidy (9) of telophase nucleus	Genes/ haploid complement
Artichoke (<i>Helianthus tuberosus</i>)	0.022 *	24	1,580	$6 \times$	260
Swisschard (<i>Beta vulgaris</i> v. <i>cicla</i>)	0.20	2.5	2,300	$2 \times$	1,150
Maize (<i>Zea mays</i>)	0.18	7.5	6,200	$2 \times$	3,100
Wheat (<i>Triticum vulgare</i>)	0.092	30	12,700	$6 \times$	2,100
Cucumber (<i>Cucumis sativum</i>)	0.96	2	8,800	$2 \times$	4,400
Onion (<i>Allium cepa</i>)	0.090	32	13,300	$2 \times$	6,650
Pea (<i>Pisum sativum</i>)	0.17	10	7,800	$2 \times$	3,900

* A 2 : 1 mixture of 1.3×10^6 and 0.70×10^6 rRNAs was used. DNA prepared from the various plant species was hybridized with 1.3×10^6 rRNA at 3 or 5 $\mu\text{g}/\text{ml}$ for 2 h in $6 \times \text{SSC}$ at 70°C . Wheat and cucumber DNAs were hybridized with pea rRNA, and the other DNAs were hybridized with their homologous rRNA. (The percentage of DNA hybridized is essentially independent of the plant rRNA^{10,11}.) The DNA of the nucleus was determined by comparative Feulgen spectrophotometry¹², on the basis that pea contained 10×10^{-12} g/telophase nucleus.

The percentage of total DNA which hybridized to rRNA varied at least forty-fold in the plants studied (Table 1). This variation was due in part to the large differences in the nuclear DNA contents of the plants. The number of copies of the rRNA gene per telophase nucleus varied from 1,600 to 13,000, but this range was increased when correction was made for the ploidy of the species and the results expressed per haploid complement, which probably corresponds in these species to the

genes per nucleolar organizer (with the exception of wheat). This assumes that all nucleolar organizers are equivalent and function, although this is certainly not known to be the case. Artichoke and swisschard contain rRNA gene redundancy similar to that observed in animal cells, whereas the redundancy in onion, pea and cucumber is much greater.

Number of Genes during Development

When the metabolic activity of plants changes, rRNA and other components accumulate at a rapid rate. Explants excised from dormant artichoke tubers depend on exogenous auxin, 2,4-dichlorophenoxyacetic acid (2,4-D), for initiation of cell proliferation¹³. Since auxin treatment increases synthesis of rRNA¹⁴, and in certain conditions exogenously supplied 2,4-D is essentially restricted to the nucleolus of the artichoke cell¹⁵, auxin stimulation of growth may involve amplification of the rRNA genes. The results in Table 2 indicate that on the

Table 2 rRNA Genes in Artichoke

Source of DNA	Initially bound DNA (µg/filter)	% Hybridization of initially bound DNA	Retained DNA (µg/filter)	% Hybridization of retained DNA
Dormant tuber	24.0	0.027	19.5	0.032
Tuber explants minus 2,4-D	25.0	0.025	14.5	0.043
Tuber explants plus 2,4-D	25.5	0.022	25.0	0.022
Young leaves	26.5	0.020	24.0	0.022

DNA was prepared from the dormant tuber tissue, from rapidly growing young leaves and from tuber explants cultured with and without 2,4-D for 3 days¹³. Thirty explants were also cultured in 3 ml. of complete medium containing 2 mCi ³H-uridine for 2 days, and total RNA was prepared. The rRNA after fractionation and elution from the gel had a specific activity of 820,000 c.p.m./µg. The DNA was hybridized to 1.3×10^6 (2 µg/ml.) plus 0.7×10^6 (1 µg/ml.) rRNA in $6 \times$ SSC at 70° C for 3 h. The values are calculated from the mean of triplicate filters, and are taken from one of duplicate experiments.

basis of the DNA initially bound to the filter there was no amplification; DNA from rapidly growing tissue (explants plus 2,4-D and young leaves) hybridized slightly less than DNA from non-growing tissue (the dormant tuber and explants minus 2,4-D). Determination of the DNA remaining on the filter after hybrid formation showed, however, that whereas DNA from growing tissues was quantitatively retained during the hybridization and washing procedure, there were large losses of the DNAs prepared from non-growing tissues. On the basis of retained DNA, the explant plus 2,4-D contained only half the number of rRNA genes present in the explant minus 2,4-D. This loss of DNA during the hybridization and washing procedure could be prevented by treating the DNA filters at 80° C *in vacuo* for 2 h⁴ immediately before hybridization, and shows that preferential loss of DNA (non-rDNA) from the filter confuses the interpretation of such experiments. A similar preferential loss of non-rDNA has been observed during hybridization at higher temperatures, and this also was prevented by the filter treatment immediately before hybridization. DNA prepared from non-growing tissue had a smaller molecular weight than that from rapidly growing material, which could account for the loss of the former DNA while the latter was quantitatively retained in comparable conditions. When the experiment was repeated with quantitative retention of the DNA, the percentage hybridization was

very similar to the initially bound DNA values in Table 2. In all other experiments, the quantitative retention of the bound DNA was ensured.

Seed germination represents a dramatic change from the dormant embryo to the rapidly growing seedling. The rapid rate of RNA synthesis during germination may require amplification of the rRNA genes. The percentage of DNA which hybridized to rRNA was, however, similar whether the DNA was prepared from the dormant embryo or from the embryo after 48 h germination, by which time the number of cells had doubled. Moreover, DNA in new cells produced during germination, from the root tip and shoot, was no different to that prepared from the complete 48 h embryo, or in those cells originally present in the dormant embryo (remainder of embryo, Table 3).

Table 3 rRNA Genes during Germination of Maize Embryo

Source of DNA	Experiment 1 1.3×10^6 rRNA 5 µg/ml.	% DNA hybridized ($6 \times$ SSC, 70° C, 2.5 hr)			
		Experiment 2 1.3×10^6 rRNA 5 µg/ml.	0.70×10^6 rRNA 2 µg/ml.	0.70×10^6 rRNA 5 µg/ml.	0.70×10^6 rRNA 2 µg/ml.
0 h embryo	0.16	0.18	0.18	0.15	—
48 h complete embryo	0.15	—	—	—	—
48 h root tip	0.19	0.17	—	0.13	—
48 h shoot	—	0.16	0.18	0.13	0.12
48 h remainder of embryo	—	0.18	0.18	0.15	0.13

DNA was prepared from embryos dissected from dry seeds, and from embryos removed after 48 h germination. The 48 h embryo was also divided into the 1 cm root tip, the shoot and the remainder of the embryo. Fifteen seeds were germinated in sterile conditions in the presence of 1 mCi/ml. of ³²P-orthophosphate for 60 h and RNA was prepared from the 1.5 cm root tips. The specific activities of the RNA used in experiments 1 and 2 were 140,000 and 19,000 c.p.m./µg respectively. The values are calculated from the means of duplicate filters.

In analogy to the animal oocyte it was necessary to consider the development of the embryo following fertilization. It was not possible to prepare sufficient DNA for hybridization from the initial stages of embryo development. However, DNA was prepared from whole ovules dissected 5 days after pollination of the wheat flower, and from embryos dissected 10 and 15 days after pollination. In terms of DNA content, the embryo was about 20% developed after 10 days, whereas by 15 days the full DNA complement was present. The DNAs from these developing tissues, from the mature embryo of the seed, and from the 3 day germinated embryo all hybridized to a similar extent with rRNA (Table 4).

These results indicate that no gross amplification of rRNA genes is associated with these phases of plant development. They do not, however, rule out amplification within a few specialized cells. The unique cytology of specialized embryo cells has been recognized for many years¹⁶, for example the giant nuclei of the suspensor cells of *Phaseolus coccineus* contain polytene chromosomes with ploidy of up to $4,096 n$ ¹⁷. However, there seems to be no information regarding specific nucleolar amplification in plant tissue. The amplification of a few specialized cells would be lost, or greatly diluted, by a large majority of unamplified cells. The problem can only be approached by either large scale micro-dissection or by quantitative *in situ* hybridization.

The results reported here contrast with those of Chen and Osborne¹⁸, who concluded that 30% of rRNA genes were deleted during the germination of wheat, and suggested that amplification occurred during development of the wheat

Table 4 rRNA Genes during Development and Germination of Wheat Embryo

Source of DNA	% DNA hybridized (3 µg/ml., 6×SSC, 70° C, 2 h)	
	1.3 × 10 ⁶ rRNA	0.70 × 10 ⁶ rRNA
Ovule, 5 days after pollination	0.101	0.066
Embryo, 10 days after pollination	0.086	0.067
Embryo, 15 days after pollination	0.096	0.069
Embryo, from dry seed	0.087	0.068
Embryo, after 3 days germination	0.089	0.068

DNA was prepared from complete ovules 5 days after pollination, from the embryos 10 and 15 days after pollination, from the embryo of the dry seed and after 72 h germination. RNA was prepared from pea roots, which had grown in the presence of 100 µCi/ml. ³²P-orthophosphate for 24 h. The specific activity of the RNA used was 58,000 c.p.m./µg, and the values are calculated from means of triplicate filters.

embryo. We saw no significant decrease in the number of rRNA genes associated with the germination of maize (Table 3) or of wheat (Table 4), and there was no evidence for gross amplification during the development of the wheat embryo (Table 4). The percentage of wheat DNA which hybridized to rRNA (0.092) agrees well with previously determined values¹¹ and is close to the value for DNA prepared from germinated wheat (0.11%) determined by Chen and Osborne¹⁸. The rRNA genes in wheat DNA, as with all the plant species so far studied, are clearly separated from the mainband DNA on CsCl fractionation¹¹, indicating that these genes are certainly clustered, probably at the nucleolar organizer, and are not distributed entirely within the chromosomal DNA, as suggested for wheat¹⁸. It was also reported that DNA preparations from germinated and ungerminated wheat embryos differed in physical properties; in particular the melting points of the

DNAs differed by 2° C¹⁸. This suggests a difference in composition of 5% G-C¹⁹, which would be a difference of 0.005 g/ml. in the buoyant densities of the two DNAs²⁰. The buoyant density of maize DNA, collected from model E analytical centrifugations over a period of 2 yr, showed no difference whether prepared from 0 h embryos (1.7009 ± 0.0005 g/ml.) or from 48 h germinated embryos (1.7012 ± 0.0006 g/ml.).

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- Perkowska, E., MacGregor, H. C., and Birnstiel, M. L., *Nature*, **217**, 649 (1968).
- Brown, D. D., and David, I. B., *Science*, **160**, 272 (1968).
- Chipchase, M. I. H., and Birnstiel, M. L., *Proc. US Nat. Acad. Sci.*, **50**, 1101 (1963).
- Gillespie, D., and Spiegelman, S., *J. Mol. Biol.*, **12**, 829 (1965).
- Wells, R., and Ingle, J., *Plant Physiol.*, **46**, 178 (1970).
- Leaver, C. J., and Ingle, J., *Biochem. J.*, **123**, 235 (1971).
- Loening, U. E., *Biochem. J.*, **102**, 251 (1967).
- Brown, D. D., and Weber, C. S., *J. Mol. Biol.*, **34**, 661 (1968).
- Darlington, C. D., and Wylie, A. P., *Chromosome Atlas of Flowering Plants* (Allen and Unwin, London, 1955).
- Matsuda, K., and Siegel, A., *Proc. US Nat. Acad. Sci.*, **58**, 673 (1967).
- Ingle, J., Possingham, J. V., Wells, R., Leaver, C. J., and Loening, U. E., *Twenty-fourth SEB Symposium: The Control of Organellar Development*, 303 (Cambridge University Press, 1970).
- McLeish, J., and Sunderland, N., *Exp. Cell Res.*, **24**, 527 (1961).
- Yeoman, M. M., Dyer, A. F., and Robertson, A. I., *Ann. Bot.*, **29**, 265 (1965).
- Key, J. L., and Ingle, J., *Sixth Intern. Conf. Plant Growth Substances* (edit. by Wightman, E., and Setterfield, G.), 711 (Runge Press, Ottawa, 1968).
- Zwar, J. A., and Brown, R., *Nature*, **220**, 500 (1968).
- Stange, L., *Ann. Rev. Plant Physiol.*, **16**, 119 (1965).
- Nagl, W., *Naturwissenschaften*, **49**, 261 (1962).
- Chen, D., and Osborne, D. J., *Nature*, **225**, 336 (1970).
- Marmur, J., and Doty, P., *Nature*, **183**, 1427 (1959).
- Sueoka, N., Marmur, J., and Doty, P., *Nature*, **183**, 1429 (1959).

Presence in Human Breast Cancer of RNA homologous to Mouse Mammary Tumour Virus RNA

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Molecular hybridization has been used to detect within human tumours RNA sequences that are homologous to the RNA of oncogenic viruses known to cause similar tumours in animals.

A VIRAL aetiology in animal cancers can be determined by inoculation of virus into the animal and the subsequent observation of cancer development. Obviously this technique can never be used in the case of human cancer, and so less direct methods must be developed to detect viral involvement in human cancer.

An accessible approach would be to determine whether human tumours contain RNA molecules homologous to a

putative human viral agent or to an analogous animal virus that is known to be oncogenic. The desirability of this approach is emphasized by the recent observation¹ in human

milk of particles similar to the mouse mammary tumour virus and the demonstration^{2,3} that these particles of human origin possess the reverse transcriptase and the 70S RNA (J. S., D. Moore, and S. P., manuscript in preparation) characteristic of the animal RNA tumour viruses. At present, the association between breast cancer and the presence of the human milk particles is not established.

Mouse Mammary Tumour Model

Molecular hybridization techniques⁴ have been successfully used with purified viruses and cells in tissue culture^{5,6}. A necessary prelude to any attempts at similar examinations of human tumours required a demonstration that these methods are applicable to animal tissues. We recently examined⁷ the technical feasibility of such experiments using mouse mammary tumours and the corresponding viral agent as an experimental model. Molecular hybridizations with radioactively labelled DNA, complementary to the RNA of the mouse mammary tumour virus (MMTV), revealed homologous RNA in both the nuclear and ribosomal fractions of the tumour cells. The results showed further that it is technically possible to identify viral-specific information in the fraction of ribosomes actively engaged in protein synthesis in a tumour cell. We report here that malignant human breast tumours were found to contain RNA molecules that exhibit homology to the RNA of the mouse mammary tumour virus but not to that of a murine leukaemia agent.

Homologous RNA in Human Breast Tumours

A number of practical and theoretical considerations led to the choice of the mouse mammary tumour virus for producing radioactively labelled DNA to search for homologous RNA in tumours. The human milk particles were not available in adequate amounts. The Mason-Pfizer agent isolated from a spontaneous mammary tumour in a rhesus monkey^{8,9} was another possibility, and experiments to be detailed elsewhere indicate that it yields results comparable with those reported here with MMTV. It has yet to be demonstrated, however, that the monkey agent actually causes tumours. Since the mouse virus is a particle of proven ability to induce breast tumours, positive outcomes with it would provide information more directly relevant to the question of viral involvement in human breast cancer.

The purification of the mammary tumour virus from the milk of the Paris RIII mouse strain^{1,2,6} and the preparation of Rauscher leukaemia virus (RLV) and the avian myeloblastosis virus (AMV) from infected plasmas⁶ have been described elsewhere. DNAs complementary to the corresponding viral RNAs were synthesized and purified as before⁶. In studies such as those with the mouse model system⁷ and with the human material described here, great care must be exercised in monitoring the purity of the DNA and RNA used. Radioactive DNA synthesized must be shown to band solely at the density of DNA in Cs_2SO_4 and hybridize to the homologous viral RNA and not to normal cellular RNA. All ^3H -DNA used in our study was subjected to these tests before use.

It is particularly important to emphasize that the alkali treatment of the DNA product must be rigorous enough to remove all RNA, as demonstrated by a proper Cs_2SO_4 profile. Any residue of the RNA·DNA hybrids formed in the reverse transcriptase reaction will make the product unusable as a probe for complementary RNA. The RNA (designated as pRNA) we used was derived from a cytoplasmic pellet consisting of a mixture of monosomes and polysomes. The RNA was purified as previously described^{6,7} with precautions to insure complete removal of protein contamination.

Our experience in searching for homologous RNA in mouse mammary tumours⁷ led us to adopt isopycnic separation in

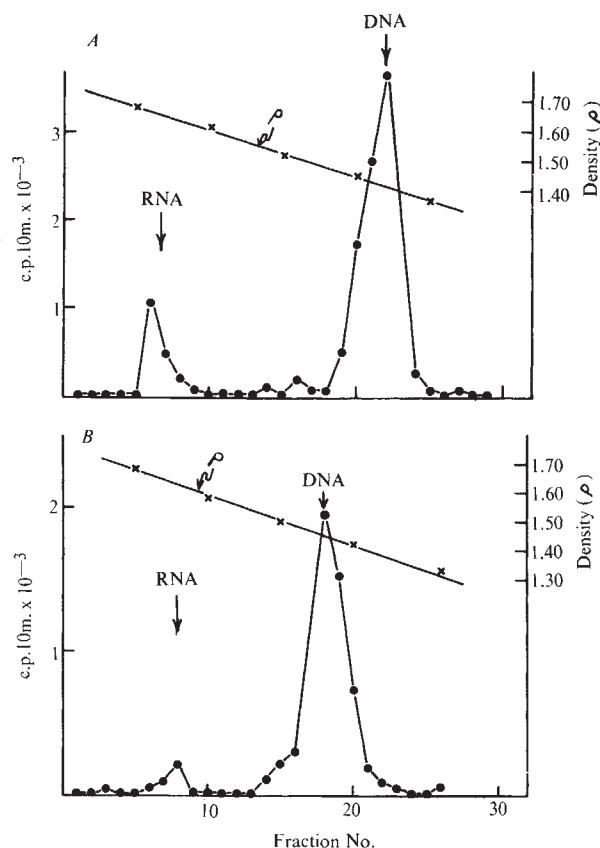


Fig. 1 Cs_2SO_4 equilibrium density gradient centrifugation of MMTV ^3H -DNA after annealing to human breast tumours C13791 (A) and 210-75-88 (B). Mouse mammary tumour virus (MMTV) was obtained from the milk of the Paris RIII strain of mice with high incidence of mammary tumours and purified as described previously^{2,6}. Synthesis of ^3H -DNA homologous to MMTV RNA was performed as follows: purified virus (1 mg protein/ml. final reaction) was preincubated in 0.2% 'NP-40' (Shell) with 80 μmol dithiothreitol (DTT) at 0°C for 10 min. The reaction mixture was then brought to a final volume of 1 ml. by the addition of 50 μmol Tris, pH 8.3, 10 μmol KCl, 8 μmol MgCl_2 , 40 μmol dGTP, dCTP, dATP, 8 μmol ^3H -dTTP at 5,000 c.p.m./pmol. The reaction was then incubated for 120 min and stopped by the addition of one-tenth volume 10% SDS and 4 M NaCl. The ^3H -DNA product was purified by phenol extraction followed by 'Sephadex G-50' chromatography to remove labelled nucleoside triphosphates and treatment with 0.4 M NaOH at 37°C for 18 h to hydrolyse any viral RNA present. pRNA (see text) was isolated from human breast tumours by disruption with a Silverson homogenizer at 4°C in 2 volumes of 5% sucrose in TNM buffer (0.01 M Tris, pH 7.4, 0.15 N NaCl, 0.002 M MgCl_2). The suspension was centrifuged at 15,000g for 40 min at 0°C . The supernatant fluid was then layered on 20 ml. of 25% sucrose in TNM and spun for 180 min at 180,000g in a Spinco 60 Ti rotor. The pellet (P-180) was resuspended in TNM and 1% SDS and the RNA extracted twice with an equal volume of cresol-phenol (pH 8.4). Nucleic acid was precipitated from the aqueous phase by the addition of two volumes of ethanol and one-tenth volume 4 M LiCl. Purified MMTV ^3H -DNA (2,000 c.p.m./reaction) was first incubated at 68°C for 10 min in 50% formamide to denature the DNA. After quick chilling to 0°C , 400 μg of C13791 breast tumour pRNA was added to A, and 400 μg 210-75-88 breast tumour pRNA was added to B. The annealing mixtures were brought to 0.4 M NaCl, 50% formamide in a total volume of 100 μl . and incubated for 18 h at 37°C . After incubation the reaction mixtures were added to 5.5 ml. of 0.005 M EDTA, mixed with an equal volume of saturated Cs_2SO_4 to yield a starting density of 1.52, and centrifuged at 44,000 r.p.m. in a 50 Ti rotor (Spinco) for 60 h at 20°C . Fractions (0.4 ml.) were collected and assayed for TCA-precipitable radioactivity.

Cs_2SO_4 gradients⁶ for the detection of hybrids. Although more laborious than other methods available, it provides the sensitivity and certainty required. In addition, all annealings were carried out at moderate temperatures (37°C) in 50% formamide to minimize thermal fragmentation of the RNA during the hybridization.

Annealing Reactions

Fig. 1 shows the sort of positive responses observed when MMTV ^3H -DNA is annealed with pRNA preparations derived from malignant tumours. Approximately 17% of the MMTV ^3H -DNA is found in the RNA density region of the gradient in one case (Fig. 1A) and 6% in the other (Fig. 1B). These profiles are to be contrasted with the negative outcomes observed when similar reactions are carried out with pRNA from normal breast tissue (Fig. 2A) and from fibrocystic disease tissue (Fig. 2B). In neither does one see MMTV ^3H -DNA in the RNA region. Fig. 3 shows a similar comparison between the responses of malignant and normal pRNA to MMTV-DNA in the form of a concentration curve. It is evident that no detectable reaction is observed with RNA derived from normal breast tissue even up to 5 mg pRNA/ml. In the same concentration range, the pRNA from malignant

tumour gives an excellent response, showing no evidence of reaching saturation at 6.5 mg RNA/ml., when about 10% of the input MMTV-DNA is complexed.

Since it is impractical to present the individual Cs_2SO_4 gradient profile of every tissue examined, a convention was adopted to permit a more convenient recording of our findings. To achieve the accuracy desired, 10 min counts (c.p. 10 m.) were taken. After correction for background counts, the sum of the ^3H counts in the RNA density region (between densities of 1.63 and 1.67 g/ml.) was used to measure the amount of DNA complexed to RNA. An operational mean background and its standard deviation (s.d.) was empirically determined by the total c.p. 10 m. counts of three tubes in the negative regions (for example, tubes 2, 3, 4) of each of fifty gradients. All specimens showing c.p. 10 m. of less than three standard deviations in the RNA density region are considered as negative. While this procedure may eliminate some positives, it provides a 99.9% confidence, and better, that those retained are significant.

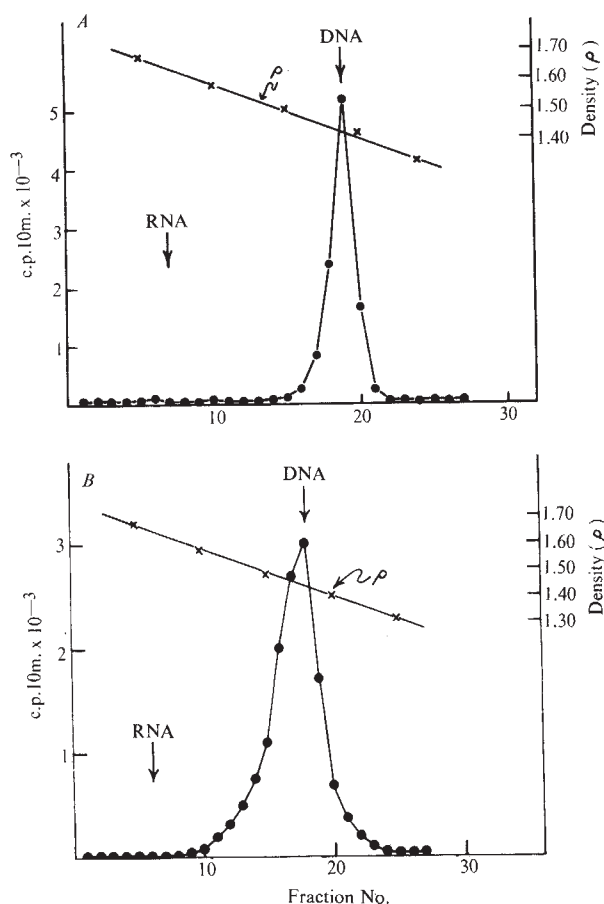


Fig. 2 Cs_2SO_4 equilibrium gradient centrifugation of MMTV ^3H -DNA after annealing to pRNA isolated from normal human breast tissue (A) and from fibrocystic disease of the breast (B). pRNA was isolated from (A) C14877 normal breast tissue and from (B) biopsy specimens of fibrocystic disease of the breast (Pool C). 400 μg of the above pRNA preparations was then annealed to purified MMTV ^3H -DNA (2,000 c.p.m./reaction). After annealing, the reactions were subjected to Cs_2SO_4 equilibrium centrifugation as described in Fig. 1.

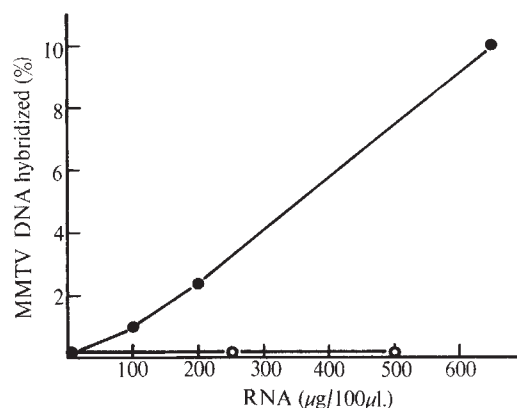


Fig. 3 Comparison of annealing reaction between MMTV ^3H -DNA, human breast tumour pRNA (●) and normal human breast tissue pRNA (○). Human breast tumour (210-75-88) pRNA and normal breast tissue (C13359) pRNA were purified and annealed to MMTV ^3H -DNA at varying RNA concentrations. The individual annealing reactions were then analysed by Cs_2SO_4 equilibrium centrifugation and the percentage DNA hybridized determined by the number of DNA c.p. 10 m. (corrected for background) banding in the RNA region (between densities 1.63 and 1.67) of the gradients.

Table 1 lists the breast tissues tested for RNA complementary to MMTV-DNA with results recorded as the total c.p. 10 m. in the RNA region and as multiples of the standard deviation. Table 2 similarly lists reactions of MMTV-DNA with pRNA derived from normal and neoplastic cells, including leukaemias and sarcomas. Fig. 4 gives a convenient pictorial summary of the results obtained.

Of the twenty-nine malignant tumours examined, 67% gave positive responses with values that can be assigned a better than one out of a thousand chance of being significant. None of the pRNA samples derived from normal breasts or the non-malignant fibrocystic and gynaecomastia tissues and fibroadenomas yielded positive reactions with the MMTV-DNA. It should be noted that actually twenty-one different samples of fibrocystic disease tissues were examined in all. The small size of the specimens usually available from patients with this disease made it necessary, except in one instance, to pool two or more in order to obtain sufficient pRNA for an adequate examination. Negative responses were also observed (Table 2 and Fig. 4) with RNA from normal human placenta, liver and intestine. Of great interest is the fact that positive

Table 1 Test for Viral-specific RNA in Human Breast Tissues

Tissue	c.p. 10 m.	c.p. 10 m./s	Reaction
Malignant adenocarcinoma			
P9	283	3.6	+
P11	419	5.2	+
P17	62	0.7	—
P21	193	2.4	—
P22	325	4.0	+
P24	436	5.5	+
C9354	375	4.7	+
C11409	372	4.7	+
C13791	1,954	24.4	+
C13809	431	5.4	+
LA-1	174	2.2	—
RU-1	113	1.4	—
HT-33	376	4.7	+
T107T	293	3.7	+
143399	120	1.5	—
24020471	458	5.7	+
210-75-88	1,257	15.7	+
5-144-FSP	230	2.9	—
8-27-FSP	208	2.6	—
9-104-FSP	59	0.7	—
9-105-FSP	510	6.4	+
9-120-FSP	370	4.6	+
14-33-FSP	290	3.6	+
17-16-FSP	83	1.0	—
17-17-FSP	428	4.3	+
24-18-FSP	537	6.7	+
24-26-FSP	40	0.5	—
40-12-FSP	278	3.5	+
Medullary carcinoma			
P5	416	5.2	+
Total tested, 29; % positive, 66			
Benign			
Fibrocystic disease			
Pool A (3)	63	0.8	—
Pool B (3)	76	0.9	—
Pool C (3)	81	1.0	—
Pool D (3)	33	0.4	—
Pool E (3)	151	1.8	—
Pool F (4)	61	0.8	—
P31	60	0.8	—
Fibroadenoma			
35T	96	1.2	—
F-1	110	1.4	—
F-2	130	1.6	—
Gynaecomastia			
9-11-FSP	106	1.3	—
Normal breast			
C13359	143	1.7	—
C14495	111	1.4	—
C14701	86	1.1	—
C14877	116	1.4	—
Total tested, 15; % positive, 0			

Results of annealing reactions between MMTV ³H-DNA and RNA isolated from human breast tissues. Specimens of benign and malignant breast tissue were obtained and frozen at the time of surgery. Diagnoses were confirmed by the final surgical pathology report. pRNA was isolated from twenty-nine carcinomas of the breast and from twenty-eight benign breast tissues, including fibrocystic disease, normal breast tissue, fibroadenomas, and gynaecomastia. From 300 to 500 µg of each sample of pRNA was annealed to 2,000 c.p.m. MMTV ³H-DNA and the reactions were analysed by Cs₂CO₄ equilibrium centrifugation. The amount of DNA banding in the RNA region of the gradient (between densities 1.63 and 1.67) was then determined. The results are expressed as c.p. 10 m. (corrected for background) banding in the RNA region for each RNA sample tested and this value divided by s.d., the operational standard deviation (see text and legend to Fig. 4). The annealing reaction is considered positive only if the c.p. 10 m. per RNA region is greater than 3S, thus providing 99.9% confidence.

responses were not observed with any of the human leukaemias and sarcomas tested (Table 2 and Fig. 4).

Specificity of Hybridization

It is evident from Tables 1 and 2, as well as from Fig. 4, that whereas 67% of the twenty-nine malignant tumours yielded RNA hybridizable to MMTV-DNA, none of the forty normal, non-malignant and irrelevant cancer tissues showed significant evidence of this type of complementary RNA. These results already argue for the specificity of the annealing reactions observed with pRNA from malignant breast tumours. Specificity can also be tested conversely. Breast tumour pRNA positive for a reaction with MMTV-DNA can be challenged with a DNA homologous to the RNA of an unrelated oncogenic virus. We have shown (S. S., R. A., R. Hehlmann, D. Kufe and J. S., manuscript in preparation) that the murine RLV-RNA and the homologous synthetic DNA do not cross-hybridize with the corresponding nucleic acids of the mouse mammary tumour agent. Consequently, RLV-DNA can be used to examine the specificity of the positive reaction observed between human breast cancer pRNA and MMTV-DNA. An example of such a test is shown in Fig. 5, in which the pRNA of tumour P5 shows a positive reaction with MMTV-DNA (Fig. 5A) and is negative (Fig. 5B) with the RLV-DNA. This and other tumour preparations positive for the MMTV-DNA reaction were tested with RLV-DNA at several pRNA concentrations with equally negative outcomes. Finally, analogous tests were done using ³H-DNA homologous to the RNA of the avian myeloblastosis virus (AMV). No breast tumour pRNA examined showed any ability to anneal to AMV-DNA.

Table 2 Reactions of Non-Breast Tumour RNA with MMTV-DNA

RNA	c.p. 10 m.	c.p. 10 m./s	Reaction
Monocytic leukaemia (M. R.)	80	1.0	—
Acute myelogenous leukaemia (G. M.)	140	1.7	—
Acute myelogenous leukaemia (W. S.)	0	0.0	—
Monocytic leukaemia (R. G.)	70	0.9	—
Osteogenic sarcoma (23-2)	20	0.3	—
Liposarcoma (1,458)	0	0.0	—
Human placenta (14 week)	80	1.0	—
Human liver	130	1.6	—
Human intestine	130	1.6	—

Results of annealing reactions between MMTV ³H-DNA and pRNA isolated from human tissue other than breast. pRNA was isolated from both malignant and normal human tissues, not of breast origin. From 300 to 500 µg of each sample of pRNA was annealed to 2,000 c.p.m. of MMTV ³H-DNA and the reaction was subjected to Cs₂SO₄ equilibrium centrifugation. The data are expressed as c.p. 10 m. (corrected for background) banding in the RNA region of the gradient and as multiples of the standard deviation (s.d.) as described in Fig. 4 and Table 1.

Concluding Comments

The negative responses to MMTV-DNA of the pRNAs from the human cancers listed in Table 2 are worthy of specific comment. When it became evident that we were obtaining positive results with MMTV-DNA in breast tumours, parallel investigations were immediately instituted with human sarcomas (D. Kufe and S. S., manuscript in preparation) and

leukaemias (R. Hehlmann and S. S., manuscript in preparation). A large proportion of the pRNA preparations from these cancers showed clear evidence of RNA homologous to the RVL-RNA but not to the RNA of either the mouse mammary tumour virus or the avian myeloblastosis virus. These results, and the negative reactions with pRNA from human leukaemias and sarcomas (Table 2), further emphasize the significance assignable to the positive reactions observed with breast tumours and MMTV-DNA.

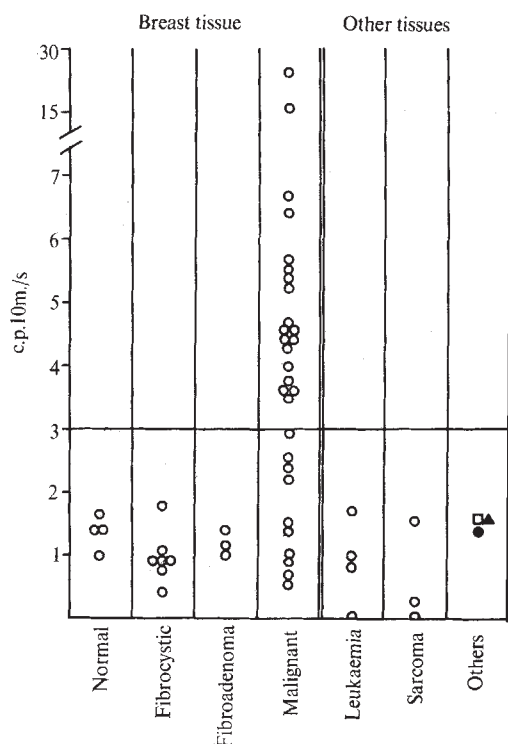


Fig. 4 Results of annealing reactions with MMTV ^3H -DNA and pRNA from breast and other tissues. Annealing reactions were performed between MMTV ^3H -DNA and pRNA isolated from specimens of normal breast, fibrocystic disease of the breast, breast fibroadenomas, gynaecomastia, carcinoma of the breast, leukaemic white blood cells from sarcomas, human placenta ($\square$), human liver ($\circ$), and human intestine ($\blacktriangle$). The reactions were then subjected to Cs_2SO_4 equilibrium density centrifugation as described in Fig. 1. The amount of ^3H -DNA, expressed as c.p. 10 m. corrected for background, banding in the density region of RNA (between densities 1.63 and 1.67) was determined for each reaction. An operational mean background and its standard deviation (s.d.) was then determined from the c.p. 10 m. of consistently negative portions (fractions 2, 3, 4) of fifty different gradient profiles. The number of DNA c.p. 10 m. (corrected for background) banding in the RNA region of the gradient was then divided by the operational standard deviation (80 c.p. 10 m.). Any reaction with c.p. 10 m. in the RNA region less than three standard deviations is considered negative, thus providing 99.9% confidence that those reactions retained as positive (greater than 3 s.d.) are significant.

It is important to recognize that the experiments described provide no measure of the extent of the homology existent between the RNA found in human breast tumour and the genome of the mouse mammary tumour virus. To obtain this sort of information will require the synthesis of MMTV-DNA in amounts adequate to saturate the relevant RNA strands in tumour pRNA.

What the experiments do imply is that there is information in the RNA of the polysome-containing fraction of human breast tumours that is specifically homologous to an animal breast tumour virus and not to other oncogenic RNA viruses.

It is unnecessary at present to over-interpret the results presented. They clearly do not constitute definitive proof of

a viral aetiology. They do, however, provide the most compelling evidence available for the involvement of virus-related information in human breast cancer. Only further experimental exploration will fully delineate the significance of these findings for the onset and development of the disease.

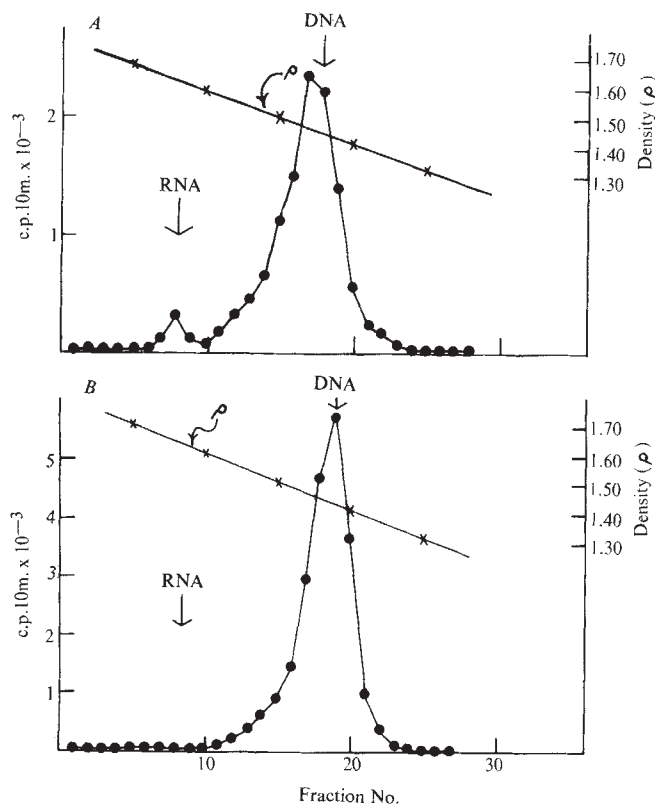


Fig. 5 Cs_2SO_4 equilibrium gradient centrifugation of MMTV ^3H -DNA (A), and RLV ^3H -DNA (B), after annealing to human breast carcinoma P5 pRNA. 400 μg of pRNA from human breast carcinoma P5 was annealed to both MMTV ^3H -DNA (A), and RLV ^3H -DNA (B), and the reactions were analysed by Cs_2SO_4 equilibrium gradient centrifugation.

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- Moore, D. H., Charney, J., Kramarsky, B., LasFargues, E. Y., Sarkar, N. H., Brennan, M. J., Burrows, J. H., Sirsat, S. M., Paymaster, J. C., and Viadva, A. B., *Nature*, **229**, 611 (1971).
- Schlom, J., Spiegelman, S., and Moore, D. H., *Nature*, **231**, 97 (1971).
- Schlom, J., and Spiegelman, S., *Science*, **174**, 840 (1971).
- Hall, B. D., and Spiegelman, S., *Proc. US Nat. Acad. Sci.*, **47**, 137 (1961).
- Green, M., Rokutanda, H., and Rokutanda, M., *Nature New Biology*, **230**, 229 (1971).
- Spiegelman, S., Burny, A., Das, M. R., Keydar, J., Schlom, J., Travnicek, M., and Watson, K., *Nature*, **227**, 563 (1970).
- Axel, R., Schlom, J., and Spiegelman, S., *Proc. US Nat. Acad. Sci.* (in the press).
- Chopra, H. C., and Mason, M. M., *Cancer Res.*, **30**, 2081 (1970).
- Schlom, J., and Spiegelman, S., *Proc. US Nat. Acad. Sci.*, **68**, 1613 (1971).

LETTERS TO NATURE

PHYSICAL SCIENCES

Cygnus X-1—a Spectroscopic Binary with a Heavy Companion?

WE have reported¹ that the spectrum, colours and interstellar features in the star HD 226868, which is coincident with the X-ray star Cygnus X-1 and a radio star^{2,3}, were those of a normal BOIb supergiant. We made measurements of the radial velocity of the star from August 1971 to October 1971 and find it to be a velocity variable, whose changes of velocity correlate with changes in the X-ray flux.

Table 1 Radial Velocity Measurements

JD	V_r	Error	Spectrograph	Dispersion	Phase
2441163.597	+25	± 25 km/s	Photographic	184 Å/mm	0.000
1165.531	+75	± 25 km/s	Photographic	184 Å/mm	0.345
1165.551	+25	± 25 km/s	Photographic	184 Å/mm	0.348
1168.440	-45	10 km/s	Photographic	62 Å/mm	0.864
1204.455	+83	10 km/s	Photographic	62 Å/mm	0.286
1206.435	-57	10 km/s	Photographic	62 Å/mm	0.639
1207.431	-50	10 km/s	Photographic	62 Å/mm	0.817
1207.444	-55	10 km/s	Photographic	62 Å/mm	0.819
1208.412	+16	10 km/s	Photographic	62 Å/mm	0.992
1225.304	+13	10 km/s	Image tube	75 Å/mm	0.004
1230.320*	-41	10 km/s	Photographic	62 Å/mm	0.899
1230.556*	-17	10 km/s	Photographic	62 Å/mm	0.943
1231.304*	+31	10 km/s	Photographic	62 Å/mm	0.074
1239.306	-25	10 km/s	Image tube	75 Å/mm	0.501
1239.321	-30	10 km/s	Image tube	75 Å/mm	0.504
1239.469	-42	10 km/s	Image tube	75 Å/mm	0.530
1255.272	+44	10 km/s	Photographic	62 Å/mm	0.349

* These spectrograms were taken by Drs K. Nandy and T. Lloyd Evans.

Our spectrograms (Table 1) were taken with the 98-inch Isaac Newton Telescope. A search among periods of a few days revealed a period of 5.60 days; the radial velocities are plotted against phase for this period in Fig. 1. Also shown is a sine curve of amplitude 64 km/s, mean 0 km/s, of arbitrary phase.

The velocity data can be interpreted as showing that the B star (of mass M_B) is in a low eccentricity orbit of inclination, i , to the plane of the sky around an object of mass m , where the mass function of the system is

$$\frac{m^3 \sin^3 i}{(m + m_B)^2} = 0.12 M_\odot$$

The projected semi-major axis of the orbit of the B star is

$$\begin{aligned} a_B \sin i &= 4.6 \times 10^6 \text{ km} \\ &= 6.6 R_\odot \end{aligned}$$

A lower limit to the mass of the companion is, from the mass function,

$$m \gtrsim 0.5 M_B^{2/3}$$

To have m less than $2 M_\odot$, we must have the mass of the BOIb star less than $6 M_\odot$, and $\sin i = 1.0$. On this interpretation of

the spectroscopic evidence alone, the system is similar to, but on the short period fringe of, other spectroscopic binaries containing blue supergiants⁴. In nearly all these systems—and especially the short period ones—the supergiant shows emission lines; emission lines are not seen in our spectra of HD 226868 (we have not examined H α). We do not see a second spectrum or even variations in the widths of the absorption lines, and would not if the companion were a normal BOV or B8I star, or fainter

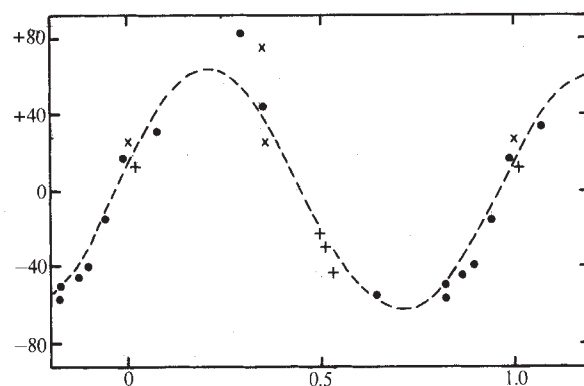


Fig. 1 The radial velocity of HD 226868 plotted against phase in a 5.60 day period. Zero phase is defined to occur at Julian Day 2441163.597. •, Measurements on photographic spectra at 62 Å/mm; x, photographic spectra at 184 Å/mm; +, image tube spectra at 75 Å/mm.

A blue supergiant rotating synchronously in a binary system of period 5.6 days has an equatorial velocity of $9.1 R_B$ km/s, where R_B is the radius of the B star in solar radii. Comparing the width of the helium lines in this star with lines in spectrograms of Slettebak and Howard's stars⁵ 55 Cygni (B3Ia, $v \sin i = 0$ km/s) and 6 Cephei (B3V, $v \sin i = 150$ km/s), we estimate a projected rotational velocity of

$$v \sin i = 100 \pm 20 \text{ km/s}$$

This observation is consistent with $R_B \sim 10 \csc i$, so that it is plausible to suggest that $10 \leq R_B \leq 30 R_\odot$ (see ref. 6) implies $0.3 \leq \sin i \leq 1.0$. A much smaller value for $\sin i$ would give an equatorial speed equivalent to escape velocity and rotational break-up.

On the spectroscopic evidence alone, we cannot entirely eliminate the possibility that the velocity variations represent an expansion and contraction of the star. The fact that the projected semi-major axis of the B star orbit is smaller than the radius of a BOIb star is reminiscent of the (incorrect) binary interpretation of the cepheid variables. The spectral type does not, however, show any of the variations with phase which one might expect if the star were changing its radius in its cycle by a factor of order two.

We have searched the X-ray data for correlations with the velocity changes. We were guided by Dolan's suggestion^{7,8}

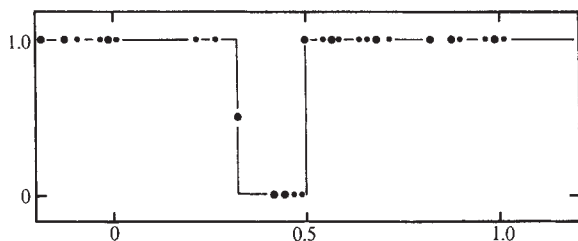


Fig. 2 Intensities of X-rays of energy above 20 keV as a function of phase for the period 5.6075 days. An intensity of 1 represents a maximum as defined by Dolan^{7,8} and 0 a minimum. The sudden drop observed by Agrawal *et al.*⁹ is the lone point at an intensity of $\frac{1}{2}$. The size of the points plotted represents the subjective opinion of the present authors, on a two-point scale, as to the reliability of the classification of the observed X-ray flux as a maximum or minimum.

that the changes in the non-pulsed intensity of the Cygnus X-1 star are attributable to its being an eclipsing binary with a period of 2.98 days. Dolan classified measurements of the X-ray flux with energy greater than 20 keV reported in the literature during the past 5 yr according to whether they were consistent with a maximum or minimum intensity. In addition, he interpreted a recent observation⁹ of a sudden drop in intensity of X-rays from Cyg X-1 as the beginning of an eclipse of one of two X-ray components in the source. We have adopted *in toto* Dolan's interpretations of the X-ray flux measurements and we plot in Fig. 2 the X-ray flux as a function of phase reduced with a period of 5.6075 days, which is also fitted by our velocity data. The phase of minimum X-ray intensity coincides with the phase of the change of velocity of the B star from positive to negative. This phase in the n th cycle occurs at times given by

$$t = \text{JD } 2441166.06 + 5.6075 n$$

Therefore, the dip in the X-radiation beam pattern (corresponding to an attenuation by a factor of about 2 over an angle of about 60°) points to Earth when the companion is between Earth and the B star.

It is this last fact which makes a simple eclipsing model for the modulation of the X-rays impossible. We can make a tentative interpretation as follows. We identify the source of the X-rays with the companion. To allow the X-radiation to pass over the B star when the companion is beyond it, the separation of the stars, d , must be such that

$$d \cos i \geq R_B$$

Coupling this condition with the mass function, we find

$$\frac{(1 + m/M_B)^{2/3}}{(0.12/M_B)^{2/3}} - \frac{(1 + m/M_B)^2}{(m/M_B)^2} \geq R_B^2/(6.6)^2$$

Using $R_B \geq 10$ and $10 \leq M_B \leq 30$ we find m to lie between 2.5 and $6.0 M_\odot$. A blue supergiant with $M_B = 10 M_\odot$ would fill the Roche lobe of such a short period orbit (see, for example, Plavec¹⁰) ejecting material to fall on to the companion and, presumably, causing non-isotropic X-ray emission. It is tempting to suppose that this interaction between the B star and its companion is connected with the X-ray fluctuations with 0.1 s timescales discovered in this source by Oda *et al.*¹¹. The small distance scale associated with fluctuations as rapid as this, and the high energy X-rays, suggest that we are not dealing with a normal main sequence or supergiant star and that the companion is a white dwarf or neutron star. The mass of the companion probably being larger than about $2 M_\odot$, it is inevitable that we should also speculate that it might be a black hole.

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- ¹ Murdin, P., and Webster, B. L., *Nature*, **233**, 110 (1971).
- ² Braes, L. L. E., and Miley, G. K., *Nature*, **232**, 246 (1971).
- ³ Rappaport, S., Zaumen, W., and Doxsey, R., *Astrophys. J. Lett.*, **168**, L17 (1971).
- ⁴ Stothers, R., and Lloyd Evans, T., *Observatory*, **90**, 186 (1970).
- ⁵ Slettebak, A., and Howard, R. E., *Astrophys. J.*, **120**, 102 (1955).
- ⁶ Allen, C. W., *Astrophysical Quantities* (second ed.) (University of London Press, 1964).
- ⁷ Dolan, J. F., *Space Sci. Rev.*, **10**, 830 (1971).
- ⁸ Dolan, J. F., *Nature*, **233**, 109 (1971).
- ⁹ Agrawal, P. C., Gokhale, G. S., Iyengar, V. S., Kunte, P. K., Manchanda, R. K., and Sreekantan, B. V., *Nature*, **232**, 38 (1971).
- ¹⁰ Plavec, M., *Bull. Astron. Inst. Czech.*, **18**, 253 (1967).
- ¹¹ Oda, M., Gorenstein, P., Gursky, H., Kellogg, E., Schreier, E., Tannanbaum, H., and Giacconi, R., *Astrophys. J. Lett.*, **166**, L1 (1971).

New Theory for Giant Loops

FOUR very large, shell-shaped features are known from the surveys of radio emission from the Galaxy. Ranging in angular diameter from 40° (Loop IV) to 116° (Loop I, the North Polar Spur), they are polarized, nonthermal radio sources that appear to be associated with faint optical emission nebulae. These objects, formerly known as the galactic spurs, have recently been reviewed by the group at Manchester which has been responsible for much of the observational and theoretical work on them¹. The term "spurs" seems to have given way to that of "loops". We prefer to call them "giant loops", because we believe that these objects represent a different phenomenon from that typified by the much smaller Cygnus Loop to which they are frequently compared.

Two principal interpretations of the giant loops are, first, that they are very large and old supernova remnants², and second, that they are caused by synchrotron emission from cosmic ray electrons moving through the galactic magnetic field in the vicinity of the Sun³. The latter theory was supported by the results of Mathewson⁴, who traced a helical component of the magnetic field in the local spiral arm, using optical polarization data on stars, and found that the pattern corresponded in part to the outlines of the giant loops. But because the giant loops are associated with filamentary H α and H β emission nebulae (see, for example, ref. 1), they cannot arise simply by synchrotron radiation from the cosmic ray gas. This point has already been noted in the case of the Cetus Arc (Loop II) by Ilovaisky and Bowyer⁵.

A number of objections have been raised to the idea that giant loops are supernova remnants (see especially Bingham⁶). The analogies drawn to the Cygnus Loop by proponents of this supernova remnant theory imply that the giant loops have linear diameters typically exceeding 170 pc and that their centres lie within a few hundred parsecs of the Sun¹. Thus, as widely noted, the supernova remnant theory is faced with the difficulty of explaining the presence of four remnants within so small a radius of the Sun. Berkhuysen *et al.*⁷ have claimed recently that this circumstance is not improbable if the giant loops are very old remnants. We note, however, that there are about 100 known remnants in the Galaxy, with diameters ranging up to 40 pc (as estimated by Milne⁸) or up to 70 pc (as estimated by Downes⁹). If the giant loops are indeed supernova remnants, where are all the remnants with diameters between 70 and 170 pc?

Shklovski and Sheffer¹⁰ considered that the giant loops, if supernova remnants, should be significant sources of soft X-rays, because their radio surface brightnesses are comparable with that of the Cygnus Loop, a known supernova remnant and soft X-ray emitter. But the observed soft X-radiation that Shklovski and Sheffer interpreted as supporting their proposal actually does not arise in the giant loops⁵. Bingham⁶ pointed out that a supernova remnant does not contain sufficient kinetic energy to become a giant loop. Although

a hypothetical "super-supernova" may be invoked⁶, as discussed by Berkhuysen *et al.*⁷ for the case of the North Polar Spur, we do not believe that we are compelled to do so. Zuzak¹¹ solves the energy problem by postulating a continuing energy source for the supernova remnant model of a giant loop, namely cosmic ray particles accelerated by a pulsar at the centre. The known pulsars¹² do not include likely candidates for the central pulsars of the four giant loops as postulated by Zuzak, but this is not a serious objection because the pulsar beams may not intersect the Earth. On the other hand, it is a very interesting coincidence that the Lupus Loop, a supernova remnant of diameter 4.5° , is located⁹ at new galactic coordinates $330.1^\circ + 15.1^\circ$, a position that agrees very well with the direction to the centre of the North Polar Spur, as given by Haslam *et al.*¹: $329^\circ \pm 1.5^\circ$, $17.5^\circ \pm 3^\circ$.

Recently, we have shown¹³ that the Gum Nebula is a fossil Strömgren sphere (FSS), that is, an H II region produced through the ionization of interstellar gas by a supernova event. We now suggest that the giant loops represent a related but much older class of nebulae, induced by supernova radiation as opposed to supernova ejecta. The Gum Nebula is probably 20,000 to 30,000 yr old, as judged from the expansion of Vela X (ref. 14). McCray and Schwarz¹⁵ have calculated the evolution of FSS on timescales up to 10^6 yr. They showed that although the central, fully ionized region is initially much hotter than the partially ionized outer parts, the centre will be cold and the outer region still hot (about 10^4 K) after 10^6 yr have elapsed. This result, the "hot ring" stage of an FSS, follows from the circumstance that the fully ionized plasma cools by radiation much more efficiently than does the partially ionized gas. The FSS, both in the hot ring stage and earlier, will obviously expand into the surrounding cooler medium. At 10^4 K, the sound speed is about 16 km s^{-1} . An Alfvén wave associated with the expansion should travel at several times this velocity. This will compress the adjacent interstellar magnetic field and cosmic ray gas, and thus will enhance the synchrotron radiation of the cosmic ray electrons. This mechanism is essentially that proposed by van der Laan¹⁶ as the source of synchrotron emission from certain supernova remnants. In this case, however, the expansion arises from the pressure difference between the FSS and its surroundings, rather than from the ejection of matter in a stellar explosion.

This model provides a qualitative explanation for the spectral and polarization properties of the radio emission from giant loops. The radio emitting electrons are part of the general cosmic ray gas and need not arise specifically in the supernova that produced the FSS. The faint optical emission nebulosities are old, partially ionized filaments, produced when the interstellar gas was ionized by the supernova. Johnson¹⁷ has independently proposed this origin as one of several possible explanations for the H β emission nebulae associated with Loop III and the Cetus Arc.

The diameters (170 pc or more) and energy contents (10^{51} ergs) of typical giant loops¹ strain the supernova remnant interpretation but are modest in terms of FSS properties^{13,15}. The estimated distances and linear diameters of the loops are probably lower limits¹. If they were somewhat larger and more distant than presently supposed, the loops would be even less likely to be supernova remnants, but could still easily be FSS. In that event, the abundance of loops near the Sun would be less than presently supposed and thus easier to explain. Another aspect of the FSS interpretation, which also relaxes the problem of the abundance of the loops, is the likelihood that FSS will have lifetimes that exceed those of supernova remnants. Berkhuysen *et al.*⁷ show that the geometry of the giant loops satisfies the supernova remnant theory, while it excludes both the helix model of Mathewson⁴ and the bubble instability process of Bingham⁶. But this test cannot distinguish between the supernova remnant and FSS models of the giant loops, because they are expected to have similar geometries.

Neutral hydrogen clouds are observed in association with

giant loops¹⁸, a circumstance which may be compatible with the supernova remnant theory⁷. In an old FSS, the denser interstellar clouds will be fully recombined and can perhaps be identified with the neutral hydrogen clouds in the loops.

We have not attempted to account for the detailed properties of giant loops, nor do we claim that our arguments are conclusive. We do believe that the radiative output of the supernova process should be looked to as a possible energy source for both the giant loops and the large scale ordering of the interstellar magnetic field that they represent.

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- ¹ Haslam, C. G. T., Kahn, F. D., and Meaburn, J., *Astron. Astrophys.*, **12**, 388 (1971).
- ² Hanbury Brown, R., Davies, R. D., and Hazard, C., *Observatory*, **80**, 191 (1960).
- ³ Tunmer, H., *Phil. Mag.*, Series 8, **3**, 370 (1958).
- ⁴ Mathewson, D. S., *Astrophys. J. Lett.*, **153**, L47 (1968).
- ⁵ Ilovaisky, S. A., and Bowyer, S., *Nature*, **233**, 470 (1971).
- ⁶ Bingham, R. G., *Mon. Not. Roy. Astron. Soc.*, **137**, 157 (1967).
- ⁷ Berkhuysen, E. M., Haslam, C. G. T., and Salter, C. J., *Astron. Astrophys.*, **14**, 252 (1971).
- ⁸ Milne, D. K., *Austral. J. Phys.*, **23**, 425 (1970).
- ⁹ Downes, D., *Astron. J.*, **76**, 305.
- ¹⁰ Shklovskii, I. S., and Sheffer, E. K., *Nature*, **231**, 173 (1971).
- ¹¹ Zuzak, W. W., *Astron. Astrophys.*, **15**, 95 (1971).
- ¹² Maran, S. P., and Modali, S. B., *Earth Extraterrest. Sci.*, **1**, 147 (1970).
- ¹³ Brandt, J. C., Stecher, T. P., Crawford, D. L., and Maran, S. P., *Astrophys. J. Lett.*, **163**, L99 (1971); Alexander, J. K., Brandt, J. C., Maran, S. P., and Stecher, T. P., *Astrophys. J.*, **167**, 487 (1971); Maran, S. P., Brandt, J. C., and Stecher, T. P., *Physics Today*, **24**, No. 9, 42 (1971).
- ¹⁴ Wallerstein, G., and Silk, J., *Astrophys. J. Lett.*, **170** (in the press).
- ¹⁵ McCray, R., and Schwarz, J., in *The Gum Nebula and Related Problems* (edit. by Maran, S. P., Brandt, J. C., and Stecher, T. P.), 60 (Greenbelt, Maryland, 1971).
- ¹⁶ van der Laan, H., *Mon. Not. Roy. Astron. Soc.*, **124**, 125 (1962).
- ¹⁷ Johnson, H. M., *H β Photometry of Galactic Nebulae at $|b| > 20^\circ$* , Lockheed Missiles and Space Company (1971).
- ¹⁸ Berkhuysen, E. M., Haslam, C. G. T., and Salter, C. J., *Nature*, **225**, 364 (1970).

Explanation of Transient Lunar Phenomena based on Lunar Samples Studies

VARIOUS authors¹⁻⁴ have shown that transient lunar phenomena (TLPs) are unlikely to arise from the excitation of luminescence in lunar rocks by such agents as the solar wind⁵, but the luminescent efficiency is many orders of magnitude too low for this emission to be detected even by "line fill" techniques⁶. To see an effect against the normal moonlight a contrast brightness change of at least 10% is necessary representing an incremental light flux from the surface of the order of $10^{-2} \text{ W cm}^{-2}$. The most likely cause of such a change is a change in the albedo. The low albedo of about 7% is a factor of 2 or 3 times lower than that of a terrestrial basaltic dust of similar composition. This is because of the "fairly castle" structure of the lunar dust, giving deep intergrain penetration of sunlight and little return except in the incidence direction. If this structure could be destroyed by breaking of the intergrain cohesion, then the albedo would rise. This could be achieved if the dust was "fluidized". Such a process has already been associated with TLPs by Mills⁷ but he assumes that the TLP is seen because of the light emission caused by electrical discharges in the venting gases between grains. But the efficiency of excitation of such discharges will be rather

low and the same kind of estimate as that made by Nash *et al.*³ for the luminescence of lunar dust will apply.

To test our hypothesis that TLPs are changes in albedo caused by dust movement or fluidization we arranged a roughened lunar dust layer on the horizontal surface of a small cup. This was mounted on an electrically driven (moving coil) vibrator working at 200 Hz and giving 0.07 mm vertical amplitude of vibration. Light is incident in a parallel pencil at a selected angle to the surface and reflectance is measured at another selected angle by a photomultiplier apertured to accept only a narrow beam of reflected light. When vibration is started the reflectance rises rapidly to a new level which can be as much as 100% above that of the static, roughened surface. The effect shows a strong dependence on viewing angle, being a maximum for an angle of 20° to the surface, this angle being relatively independent of the incidence angle. But the latter does affect the magnitude of the increase in albedo. We would expect the maximum value at about 20° because grain to grain shadowing will be highest around that angle. Fig. 1 gives polar plots of normal and fluidized dust data for reflectance at two different angles of incidence. Fig. 2 gives a typical set of diffuse reflexion spectra for incident light 30° to the normal to the surface and reflected light at 30° to the normal on the opposite side from the incident direction. No significant colour change occurs on fluidization but we need more data for other angles of incidence and reflexion to see if this is generally true.

Such angular dependences of change in albedo caused by dust flow show that TLPs have favourable viewing times. We are at present reviewing observation times and surface locations of reported TLPs to see whether they conform to our expected conditions of maximum contrast visibility.

Another transient phenomenon in the solar system has been the temporary brightening of the zodiacal light for some hours and at times after solar flares, consistent with the arrival of an incremental solar wind pulse ($\sim 10^{-7}$ W cm⁻²) at the meteoric dust belt which, by scattering sunlight towards the Earth, gives rise to the zodiacal light. For reasons already established for lunar samples and also for stone meteorites, the transient brightness increase cannot be the result of excitation of luminescence by the solar wind. Moreover, the light scattering zodiacal belt particles are at a greater distance from the Earth than the lunar surface by an order of magnitude. The most likely cause of the observed increase would, however, be a rise in scattering intensity attributable to some increase in preferred orientation of irregularly shaped particles of dust

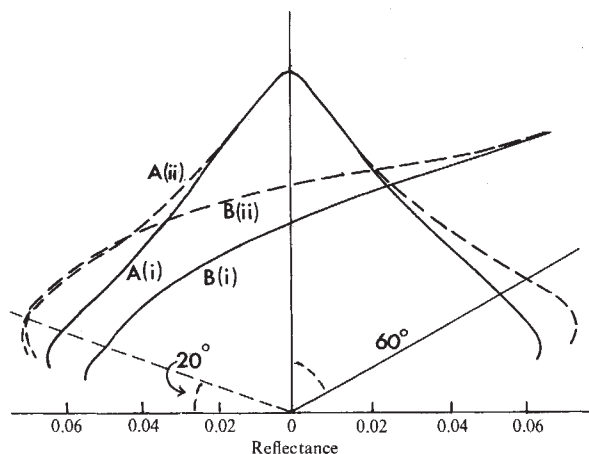


Fig. 1 Variation of Apollo 14 lunar dust sample reflectance (sample 14259.56) with viewing angle for two different angles of incidence and under static and "fluidized" conditions. *A*, Incidence angle 0° to normal: (i) static dust layer, (ii) "fluidized" dust layer. *B*, Incidence angle 60° to normal: (i) static dust layer, (ii) "fluidized" dust layer. The broken line shows viewing angle for maximum brightening effect.

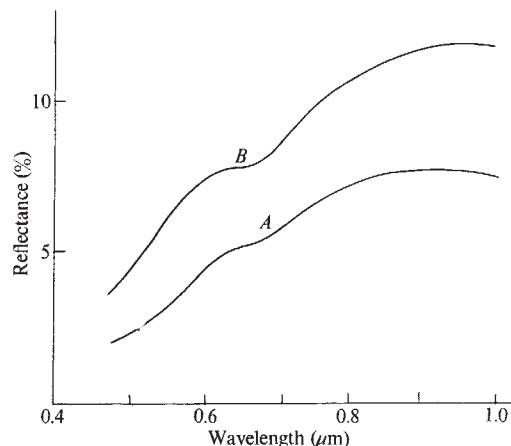


Fig. 2 Diffuse reflexion spectra of a lunar dust sample (14259.56) in static and fluid conditions measured with incident light at 30° to normal and reflected light at 30° to opposite side of normal. *A*, Static dust layer; *B*, fluidized dust layer.

in a uniaxial stream of atomic particles with which momentum could be exchanged. This is a plausible but a more tentative hypothesis than the corresponding explanation of TLPs but it is capable of laboratory simulation. We are now looking into the design of such an experiment.

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- ¹ Moore, P., *J. Brit. Astron. Assoc.*, **81**, 365 (1971).
- ² Geake, J. E., Dollfus, A., Garlick, G. F. J., Lamb, W. E., Walker, G., Steigmann, G. A., and Titulaer, C., *Proc. Apollo 11 Lunar Sci. Conf.*, 2127 (Pergamon, New York, 1970).
- ³ Nash, D. B., and Greer, R. T., *Proc. Apollo 11 Lunar Sci. Conf.*, 2341 (Pergamon, New York, 1970).
- ⁴ Blair, I. M., and Edgington, J. A., *Proc. Apollo 11 Lunar Sci. Conf.*, 2001 (Pergamon, New York, 1970).
- ⁵ Kopal, Z., and Rackham, T., *Icarus*, **2**, 481 (1963).
- ⁶ Grainger, J. F., and Ring, J., *Proc. Intern. Space Sci. Symp.*, 989 (1963).
- ⁷ Mills, A. A., *Nature*, **225**, 929 (1970).

Structure of the Fe-S Complex in a Bacterial Ferredoxin

BACTERIAL ferredoxin was isolated from *C. pasteurianum* by Mortenson¹ and characterized as a nonhaem iron protein. The term ferredoxin was subsequently broadened to cover other iron-sulphur proteins including those involved in the photosynthetic process in chloroplasts. All ferredoxins have similar electron paramagnetic resonance (EPR) spectra and very low E_0 values in the neighbourhood of -0.4 V.

The type ferredoxin isolated by Mortenson is sometimes referred to as clostridial ferredoxin. It is characterized by the presence of eight Fe atoms and eight labile S atoms (inorganic) along with eight cysteine S atoms per molecular weight of approximately 6,000². We report here the structure of the clostridial ferredoxin isolated from *Micrococcus aerogenes*.

The crystals are orthorhombic with unit cell parameters $a = 30.52$ Å, $b = 37.75$ Å, $c = 39.37$ Å. The space group is $P2_12_12_1$ (ref. 3). The arrangement of the Fe and S atoms in the clostridial ferredoxins is a problem of considerable in-

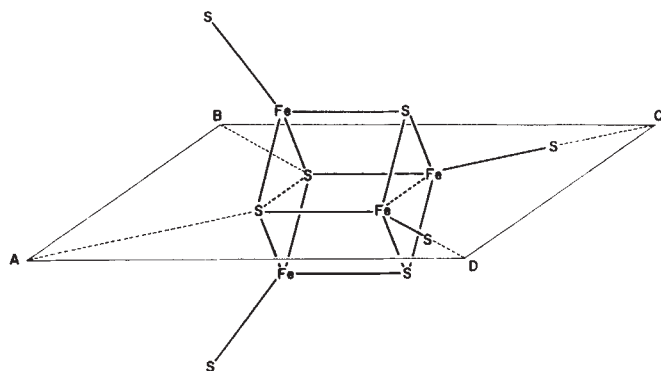


Fig. 1 Model of a 4Fe-8S cluster.

terest. A number of models have been proposed, but none is consistent with Patterson maps at resolutions of 5 Å, 3.5 Å, and 2.5 Å. It proved impossible to deduce even tentative positions of the Fe atoms from the Patterson map. Unsuccessful attempts were also made to locate the Fe atoms from various difference Patterson syntheses in which coefficients dependent on Fe scattering were used such as Bijvoet differences⁴ for CuK α data from native crystals and differences in the real and imaginary components of the scattering factor for CuK α and CoK α radiations.

The direct determination of the phases of the X-ray reflexions by the method of multiple isomorphous replacement⁵ is the most powerful method of solving a protein crystal structure.

Table 1 Refinement Criteria*

	R_1	R_2	R_K	R_{CuII}	$\langle E \rangle$	$\langle F_H' \rangle$	$\langle m \rangle$	n
Sm ⁺³	43.7	5.5	8.2	47.8	19	40	0.64	1,115
Pr ⁺³	43.4	5.0	7.1	47.1	19	40	0.63	760
UO ⁺²	44.1	8.8	13.0	48.8	35	72	0.61	730

$$R_1 = \frac{\sum_{hkl} ||F_H| - |f_H||}{\sum_{hkl} |F_H|}$$

where F_H is observed heavy atom contribution calculated according to Mathews¹⁶. f_H is calculated structure amplitude of heavy atoms.

$$R_2 = \frac{\sum_{hkl} ||F_{PH}| - |F_P + f_H||}{\sum_{hkl} |F_{PH}|}$$

where F_{PH} is observed structure amplitude of derivative. F_P is observed structure amplitude of native protein.

$$R_K = \frac{\sum_{hkl} ||F_{PH}| - |F_P + f_H||}{\sum_{hkl} ||F_{PH}| - |F_P||}$$

for data from centrosymmetric projections.

$$R_{CuII} = \frac{\sum_{hkl} ||F_{PH}| - |F_P + f_H||}{\sum_{hkl} ||F_{PH}| - |F_P||}$$

for data from centrosymmetric projections.

$$\langle E \rangle = \left\{ \frac{\sum (|F_{PH}| - |F_P + f_H|)^2}{n} \right\}^{\frac{1}{2}}$$

evaluated at the "best" phase and n is the number of observed reflexions.

$$\langle F_H' \rangle = \left\{ \frac{\sum (|F_{PH}| - |F_P|)^2}{n'} \right\}^{\frac{1}{2}}$$

where n' is the number of reflexions.

$\langle m \rangle$ = mean figure of merit. All F_s , E_s and so on are on a relative scale consistent with each other.

Many heavy atom reagents that have given satisfactory derivatives with other proteins by the soaking method were tried without success. The difficulty was due in part to the labile Fe-S complex and the general instability of the protein in the presence of oxygen. Those reagents which ultimately proved successful were UO₂(NO₃)₂, Sm(NO₃)₃, and PrCl₃, all in relatively high concentration.

CoK α radiation ($\lambda = 1.7902$ Å) was used for four sets of data: native crystals and Sm⁺³ derivative with 2.5 Å < d , UO₂⁺² and Pr⁺³ derivatives with 3.0 Å < d . Both reflexions of each Friedel pair were measured so that the effects of anomalous scattering could be used to resolve the phase ambiguity of a single derivative⁶. CoK α radiation was used instead of CuK α because of the much smaller imaginary component of the Fe scattering factor.

The difference Patterson for the Sm⁺³ derivative was readily interpreted in terms of two main sites, about half occupied, and two minor sites. The two major sites and one of the six minor sites of the Pr⁺³ derivative are in essentially the same positions as the two major sites and one of the minor sites in the Sm⁺³ derivative. The difference Patterson for the UO₂⁺² derivative was interpreted independently of the other two derivatives and led to a model with eleven partially occupied sites and with no one site dominating the model. Phases were determined independently for each derivative by use of anomalous scattering data to resolve the phase ambiguity. Refinement criteria are summarized in Table 1.

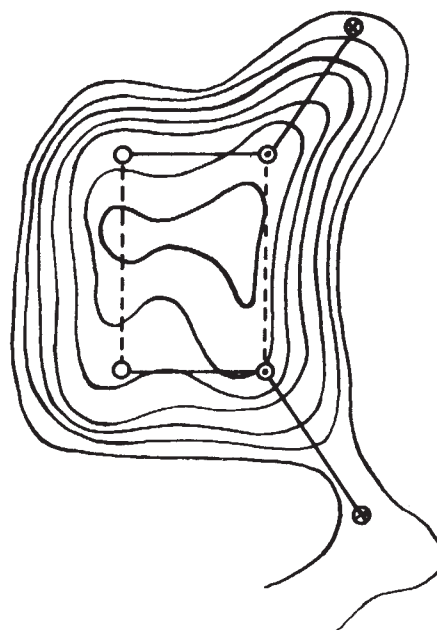
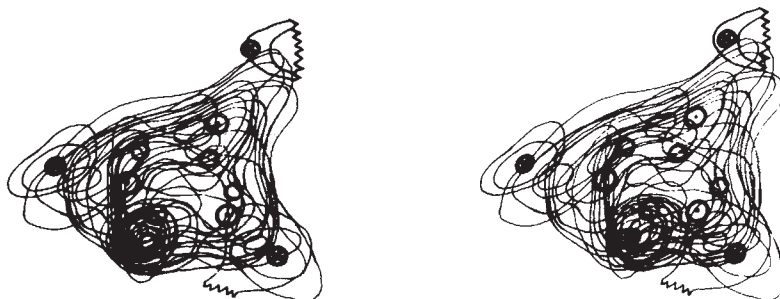


Fig. 2 Section of electron density through one Fe-S cluster from map calculated with phases based on Sm⁺³ derivative. Atoms of model in this section identified as follows: ○, Fe; ⊗, cysteine S; ○, inorganic S.

Three "best"⁷ electron density maps, one for each derivative, have been computed. All three maps agree in the main features of the molecule, but the 2.5 Å resolution map based on the Sm⁺³ derivative shows more detail than the other two at 3 Å resolution. The outlines of the ellipsoidal molecules are evident in each map. Within the molecule, two clusters of outstanding electron density separated by a distance of 12 Å can be clearly seen. Both clusters show four lobes projecting in a tetrahedral fashion from alternate corners of a roughly cube-shaped region of highest density. Both clusters seem to be identical in structure and can be fitted rather well by a model of four Fe and four S atoms at alternate corners of a cube with four more S atoms projecting from the iron atoms (Fig. 1). This is essentially the same arrangement as that reported for

Fig. 3 Stereo view of one Fe-S cluster. Direction of view inclined to the normal of plane ABCD in Fig. 1. Since atomic positions are on sections of the electron density map, the coordinates normal to the sections are subject to errors up to 0.5 Å (half the grid spacing). Identification of atoms as in Fig. 2.



the Fe-S complex in high potential iron protein (HiPIP) from chromatium⁸⁻¹¹. That the cysteine S atoms correspond to the four lobes of each of the ferredoxin clusters follows from the fact that chains of electron density, corresponding to the main chain of the protein, are attached to each lobe.

Assuming an Fe-S distance of 2.3 Å, a value near that found in the protein rubredoxin¹² and in other iron-sulphur structures^{13,14}, a good fit to a section of the electron density close to the plane ABCD of Fig. 1 can be demonstrated (Fig. 2).

Fig. 3 is a stereo view of the same cluster from which the section in Fig. 2 was taken. All four lobes of the cluster are visible although one of them on the lower left extends down away from the viewer and is partly obscured by the contours of the central high-density region.

The similarity in structure of the Fe-S clusters in ferredoxin with the complex in HiPIP raises some questions of great interest in view of the different properties of the two molecules. For example, why are the E_0 values so different, that for HiPIP being +0.3 V as compared with the ferredoxin value of -0.4 V? To what extent are the properties of the iron-sulphur complex modified by the surrounding protein chains and side groups? To what extent do distortions of the complex modify its properties and are such distortions dependent on the rest of the molecule? In this context it should be observed that chloroplast ferredoxins with very different Fe and S content (two Fe and two labile S atoms per molecule) have properties such as E_0 values and EPR spectra similar to those of the clostridial ferredoxins.

From our electron density map based on the Sm^{+3} derivative, much of the main chain in the molecule can be traced, but there are regions where it is uncertain. Improved phases obtained by combining the derivatives at hand should remove the uncertainty about the path of the protein chain. Moreover, knowledge of the Fe and S positions along with anomalous scattering data for CuK_α radiation can also be used to improve the phases. The amino-acid sequence for this protein has already been determined and will be of great value in elucidating the structure¹⁵.

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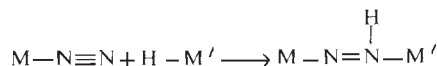
Received September 6; revised October 15, 1971.

- ¹ Mortenson, L. E., Valentine, R. C., and Carnahan, J. E., *Biochem. Biophys. Res. Commun.*, **7**, 448 (1962).
- ² Hong, J. S., and Rabinowitz, J. C., *J. Biol. Chem.*, **245**, 4982 (1970).
- ³ Sieker, L. C., and Jensen, L. H., *Biochem. Biophys. Res. Commun.*, **20**, 33 (1965).
- ⁴ Rossmann, M. G., *Acta Cryst.*, **14**, 383 (1961).
- ⁵ Green, D. W., Ingram, V. M., and Perutz, M. F., *Proc. Roy. Soc. A*, **225**, 287 (1954).

- ⁶ Blow, D. M., and Rossmann, M. G., *Acta Cryst.*, **14**, 1195 (1961).
- ⁷ Blow, D. M., and Crick, F. H. C., *Acta Cryst.*, **12**, 794 (1959).
- ⁸ Kraut, J., Strahs, G., and Freer, S. T., *Structural Chemistry and Molecular Biology*, **54** (1968).
- ⁹ Strahs, G., and Kraut, J., *J. Mol. Biol.*, **35**, 503 (1968).
- ¹⁰ Carter, C. W., Freer, S. T., Xuong, Ng H., Alden, R. A., and Kraut, J., *Cold Spring Harbor Symp. Quant. Biol.*, **36** (in the press).
- ¹¹ Phillips, W. D., Poe, M., McDonald, C. C., and Bartsch, R. G., *Proc. US Nat. Acad. Sci.*, **67**, 682 (1970).
- ¹² Watenpugh, K. D., Sieker, L. C., Herriott, J. R., and Jensen, L. H., *Cold Spring Harbor Symp. Quant. Biol.*, **36** (in the press).
- ¹³ Schunn, R. A., Fritchie, C. J., and Prewitt, C. T., *Inorg. Chem.*, **5**, 892 (1966).
- ¹⁴ Wei, C. H., Wilkens, G. R., Treichel, P. M., and Dahl, L. F., *Inorg. Chem.*, **5**, 900 (1966).
- ¹⁵ Tsunoda, J. N., Yasunobu, K. T., and Whiteley, H. R., *J. Biol. Chem.*, **243**, 6262 (1968).
- ¹⁶ Matthews, B. W., *Acta Cryst.*, **20**, 230 (1966).

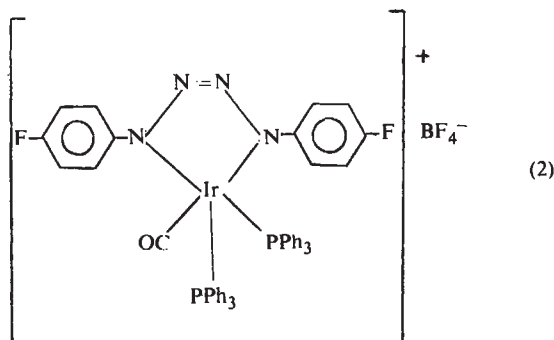
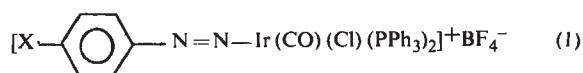
Transition Metal Complexes of Diazonium Salts as Models for Nitrogenase

THE recent synthesis of a wide variety of transition metal complexes of molecular nitrogen (dinitrogen) has underlined the strong possibility that formation of a molybdenum—or iron—dinitrogen complex is an initial step in biological nitrogen fixation. Furthermore, complexes have been synthesized in which the dinitrogen ligand bridges two transition metals. These exhibit a substantial reduction in the N-N stretching frequency, $\nu(\text{N}_2)$, and it may well be that dinitrogen is activated by bridging iron and molybdenum in the metallo-enzyme itself¹. Specifically, Chatt has suggested that dinitrogen is probably taken up initially at the iron site, to form a dinitrogen complex of rather low $\nu(\text{N}_2)$, which is lowered still further by the attachment of the molybdenum to the free end of the dinitrogen ligand, and thereby made more susceptible to facile reduction. An extension of this hypothesis is that of R. W. F. Hardy and E. Knight who suggest (personal communication) that one metal may be present as a hydride, in which case the following intermediate would result from insertion of the dinitrogen complex into the metal hydride bond:

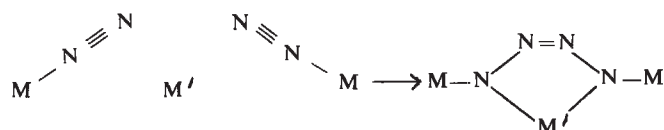


Parshall² has demonstrated that aryldiazonium salts, where the $\text{Ar}-\text{N}\equiv\text{N}^{\oplus}$ function serves as a model for the $\text{M}-\text{N}\equiv\text{N}$ complex, will indeed insert into the $\text{H}-\text{Pt}$ bond in $\text{Pt H Cl}(\text{PEt}_3)_2$ and other similar reactions have been reported³. Now, recent studies in our laboratory on the reactions of *p*-substituted aryldiazonium salts with Vaska's complex, $\text{trans-Ir Cl}(\text{CO})(\text{PPh}_3)_2$, have yielded two novel compounds both of which are relevant to current theories of nitrogen fixation.

The anticipated product of the reaction is (1); however, we have not found this to be present.



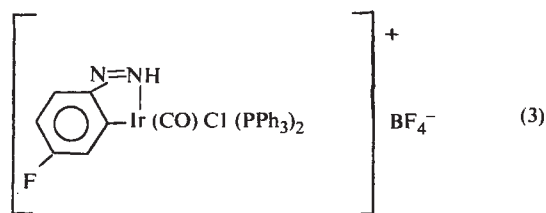
Instead, crystals of the tetrazene complex (2) were obtained directly from the reaction mixture⁴. Formally, the tetrazene moiety can be viewed as resulting from the coordination of two diazonium groups to the iridium through their bound nitrogen atoms, with linking together of their free nitrogen atoms. Translating this result literally to the nitrogen fixation process would require invoking a new model in which two dinitrogen moieties bound on similar adjacent sites are activated by simultaneous binding to a third, different metal through the 1 positions, and linked together by the 2 positions. The lowering of N-N bond strength inherent in such a model would be considerable.



In our complex, X-ray crystallography shows that the central bond length corresponds to an N=N double bond and the outer bond lengths approximate to single bonds⁴, whereas these were triple bonds in the original diazonium salt. Little attention seems to have been given to the possibility of "poly-

merization" of the dinitrogen molecule occurring in nitrogen fixation, although Shustorovich⁵ has entertained this possibility on theoretical grounds.

The second product, isolated from the mother liquor, is the yellow crystalline compound (3). Its composition has been determined unambiguously by an X-ray structure determination which will be reported separately.



This compound differs from the anticipated product (1) only in that the coordination sphere of Ir has been expanded to six by the apparent migration of the *ortho* proton and coordination of the *ortho* carbon atom to Ir. There are two important features of this compound to be noted in connexion with the nitrogenase model.

(1) $\nu(\text{N}_2)$ occurs at $\sim 1,415 \text{ cm}^{-1}$ ($\text{X}=\text{F}$) which represents a substantial lowering from the value ($\sim 2,300 \text{ cm}^{-1}$) in the diazonium salt, exceeding the lowering of $\nu(\text{N}_2)$ observed for some complexes with bridging dinitrogen ligands¹.

(2) Spontaneous hydrogenation of the nitrogen function has occurred as a result of the rearrangement induced by the tendency of Ir(III) to achieve six-coordination by bonding with the *ortho* carbon atom of the aryl ring. Thus, the equivalent of the diimide complex in Parshall's model has been achieved in our system without the participation of a metal hydride. Furthermore, complex (3) undergoes further hydrogenation under extremely mild conditions (H_2 , 1 atm, 25°C , Pd/BaSO₄) to yield the hydrazine complex (4).

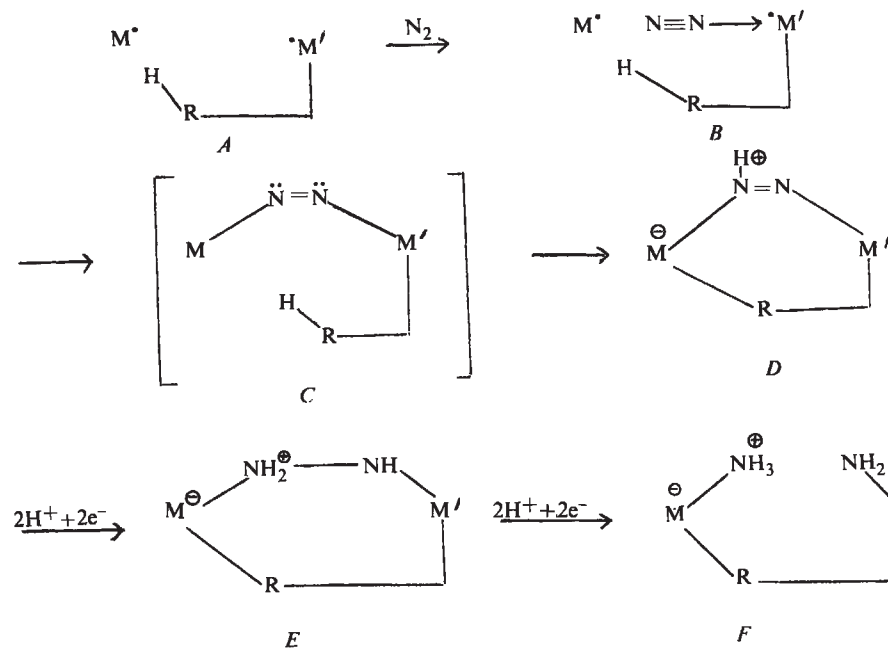
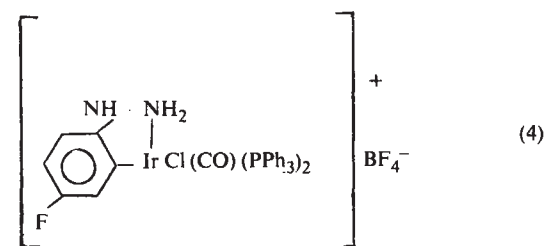
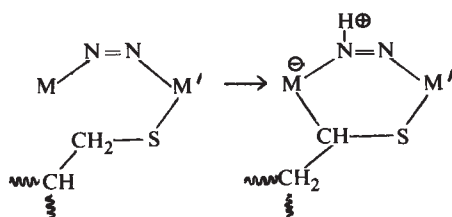


Fig. 1 M^* , M' do not necessarily signify metals possessing an impaired electron, but signify the necessary availability of two non-bonded electrons required for the electron rearrangement $\text{B} \rightarrow \text{C}$.

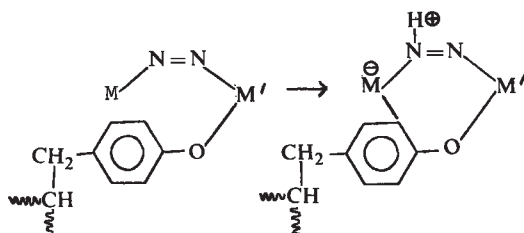
We therefore wish to direct attention to the strong possibility that in nitrogenase, the initial hydrogenation of the complexed dinitrogen molecule to a diimide complex arises from a rearrangement involving C-H bond cleavage, rather than the involvement of a metal hydride. There are now numerous examples of transition metal complexes involving coordination from the *ortho* carbon atom of an aromatic ring⁸ or from a methylene group⁹. In all examples till now, however, the eliminated hydrogen atom has either migrated to the metal to form a hydrido-complex, or has been removed completely, for example, as hydrogen halide⁶⁻⁸.

A suggested scheme is shown in Fig. 1. Here we envisage that M' is bonded to a group RH which is oriented in close proximity to M. Coordination of dinitrogen and electron rearrangement to the intermediate C is followed by proton shift to give the diimide complex D. An important feature in the activation of the dinitrogen molecule towards further reduction would be the possibility of electron delocalization in the resulting cyclic complex leading to a reduced N=N bond order.

Reduction by 6e⁻ would lead directly to the formation of NH₃ and regeneration of A. There are a number of obvious groups present in the enzyme which might fill the role of R-H in this model; as two examples, the conversion C→D could involve a cysteine residue:



or a tyrosine residue:



M and M' are most likely to represent the two metals, iron and molybdenum, necessary for nitrogen fixation. It should be kept in mind, however, that the ability to consume atmospheric nitrogen is not restricted to metallic systems, since two instances of organic compounds exhibiting such behaviour are known^{9,10}. Furthermore, dinitrogen is capable of bridging between a metal and a non-metal (for example, phosphorus) to give complexes with similar values of $\nu(\text{N}_2)$ to the purely metal bridged complexes¹. It would therefore be premature to rule out the possibility that dinitrogen is attached to a non-metal in the enzyme¹¹. Thus, in the above examples, it is instead conceivable that the dinitrogen is attached directly to the sulphur atom of the cysteine or to the oxygen of the tyrosine residue, without intervention of M'. The model would still apply, and the ring size would be smaller.

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1. Chatt, J., Dilworth, J. R., Richards, R. L., and Saunders, J. R., *Nature*, **224**, 1201 (1969).
2. Parshall, G. W., *J. Amer. Chem. Soc.*, **89**, 1822 (1967).
3. Toniolo, L., and Eisenberg, R., *Chem. Commun.*, 455 (1971).
4. Einstein, F. W. B., Gilchrist, A. B., Rayner-Canham, G. W., and Sutton, D., *J. Amer. Chem. Soc.*, **93**, 1826 (1971).
5. Shustorovich, E. M., *J. Struct. Chem. (USSR)*, **11**, 154 (1970).
6. Parshall, G. W., *Accounts Chem. Res.*, **3**, 139 (1970).
7. Cheney, A. J., Mann, B. E., Shaw, B. L., and Slade, R. M., *Chem. Commun.*, 1176 (1970).
8. Cope, A. C., and Siekman, R. W., *J. Amer. Chem. Soc.*, **87**, 3272 (1965).
9. Owsley, D. C., and Helmkamp, G. K., *J. Amer. Chem. Soc.*, **89**, 4558 (1967).
10. Ellesmann, J., Poersch, F., Kunstmann, R., and Kamolowsky, R., *Angew. Chem., Intern. Ed.*, **8**, 203 (1969).
11. Newton, W. E., Corben, J. L., Schneider, P. W., and Bulen, W. A., *J. Amer. Chem. Soc.*, **93**, 268 (1971).

BIOLOGICAL SCIENCES

X-ray Diffraction from Outer Segments of Visual Cells in Intact Eyes of the Frog

X-RAY diffraction patterns have been obtained from the light receptor membranes of frog retinal rod outer segments (of *Rana temporaria* and *pipiens*)^{1,2}, and a one dimensional electron density profile across the membrane has been derived¹. Until now the closest approach to studying the membranes in physiological conditions has been to pass X-rays through the outer segment layer of a strip of retina dissected from an eye and placed in a chilled glucose-supplemented Ringer solution¹. To obtain a higher resolution electron density profile and minimize the possibility of artefact production, I have recorded X-ray diffraction patterns from photoreceptor layers in intact isolated whole eyes and also from eyes in a pithed frog with its heart beating throughout the experiment. Insofar as the respiratory system keeps functioning and the choroid and retina adjacent to the outer segments are intact, this experiment approaches an *in vivo* study.

A small strip of the outer wall protecting the back of the eye—the sclera—was cut away leaving the outer segments insulated from the air by the choroid, the Bruch's membrane and the pigment epithelium^{3,4}. Frogs, chilled in ice-water before pithing, were kept near 0° C when exposed to X-rays in an atmosphere of wet air (100% relative humidity) whilst chilled glucose-supplemented Ringer's solution was dropped onto the choroid. Elliott toroidal type⁵ and Franks type⁶ point focusing X-ray cameras were used. Copper K- α X-rays were provided by Hilger-Watts microfocus generators and also by an Elliott rotating-anode generator. X-rays were passed tangentially to the surface of the choroid at a depth sufficient to intercept the outer segments. Replacing the Ringer solution by water had no detectable effect on the periodicity in the osmotically sensitive outer segments⁷. In cases where the choroid was not kept moist, or where the retina had been accidentally punctured, a characteristic diffraction pattern was recorded, during which the orientation of the diffraction orders rotated (compare Fig. 1a and b). On removal of the front of the eye of a frog that had been X-rayed overnight, as far as could be seen, the vitreous humour and retina were transparent and free from opacities.

The intensity distributions of the lamella diffraction patterns obtained in this laboratory by Blaurock and Wilkins from outer segments in a slice of excised retina are largely confirmed by this study. In addition the orientation of the diffraction pattern shows that the long axes of the outer segments are oriented towards the lens and not towards the centre of the

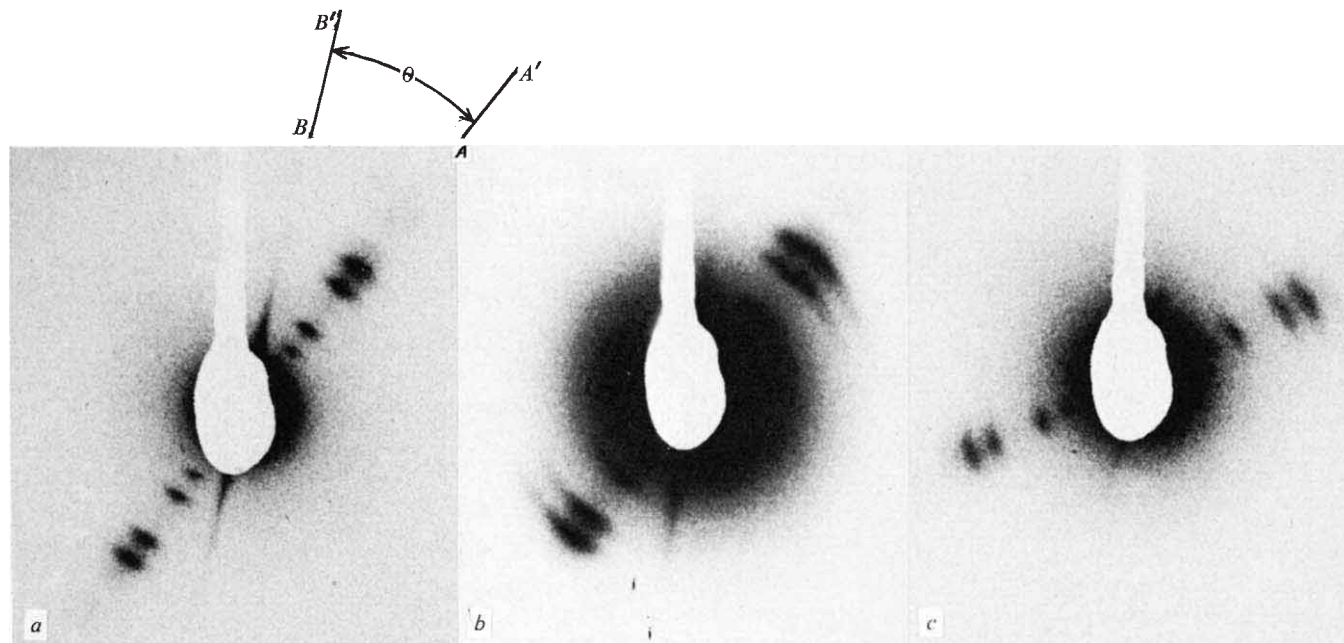


Fig. 1 *a*, Diffraction pattern of retinal rod outer segment membranes in a pithed frog, showing pairs of orders 3, 4, 6 and 7 of a $295 (\pm 5)$ Å interdisk periodicity oriented along AA' . Orders 0, 1 and 2 and the undiffracted beam are obscured by the beam stop. The dark spikes radiating from the centre along BB' are oriented perpendicular to the surface of the eye and may arise from the choroid-air interface. Hence the angle θ between AA' and BB' is the average tilt of the rods away from the perpendicular to the retina surface. Specimen-to-film distance $12.8 (\pm 0.2)$ cm. Exposure time 1.75 h. Rotating-anode generator and toroidal camera. *b*, Diffraction pattern from excised whole eye showing signs of drying. The photograph has been overexposed during processing to show the rotation of the pattern. This is often accompanied by a change in the relative intensities of the diffraction orders and the periodicity alters and becomes less sharply defined. Possibly the rods fall over or the whole retina rotates while the eye collapses. Period initially $304 (\pm 5)$ Å. Exposure time 22 h. *c*, Skewed diffraction pattern—interpreted in the text and in Fig. 2. Although the tilt is greater than in *a*, no correlation between tilt and skewed arcs is apparent, for similar dissections on the same part of the retina sometimes give normal and sometimes skew patterns. Exposure time 4.5 h. Period $304 (\pm 5)$ Å.

eye. This observation on unfixed eyes agrees with studies by light microscopy of fixed and sectioned whole eyes^{8,9}. The orientation might be expected since the outer segments maximally absorb light incident along their long axis¹⁰, and also the spatial resolution of the eye is optimized. Actually, it is the orientation of the direction of stacking of the outer segment disk membranes that is observed by X-ray diffraction. It is assumed here, in accordance with polarized light and electron microscopy studies^{10,11}, that on average the disk membranes are stacked parallel to the long axis of the outer segments.

Stiles has reviewed the evidence showing that *in situ* in human and frog eyes, cones are sensitive to the angle at which light strikes the retina whereas rods are not¹². Rods would lack this sensitivity if they were disoriented over a solid angle comparable with that subtended by the dark-adapted pupil at the retina; but this seems an inefficient way of catching light. This degree of disorientation has not been found—the observed arcing of the diffraction spots is often comparable to the much smaller angular size of the light-adapted pupil. Therefore the lack of angular sensitivity of rods in the frog retina is not due to their disorientation. Instead the angular field of sensitivity of rods must be wider than that of cones.

An unusual feature of many diffraction patterns is shown in Fig. 1*c*: the diffraction arcs appear skew if compared with those in Fig. 1*a*; that is, one side of all the arcs is nearer to the centre of the X-ray pattern. The possibility that the X-ray camera is misaligned has been checked and eliminated. The skewness is interpreted as a combination of a variation in the specimen-to-film distance, due to the size of the eye, with an asymmetric angular disorientation of the rods about their average orientation towards the lens. The asymmetric disorientation has been found, so far, only on the equator of the eye, corresponding to peripheral vision. On the top and bottom of the peripheral retina diffraction patterns like that in Fig. 1*c* have been found. The patterns are consistent with the interpretation that at each point along the retina at which

the path of the X-rays is intersecting retinal rods, the disorientation of the photoreceptor membranes is least in the plane which also contains the optic axis of the lens (Fig. 2). More detailed analysis first requires further checks to be made in case removing the sclera affects the disorientation by asymmetrically stressing the retina.

The average orientation of the photoreceptor membranes is that which catches most light. One may ask how the membranes become thus oriented, especially as a new disk forms about every 40 min in the frog¹³. It seems inefficient to specify genetically the unique orientation found at each point of the retina: I conjecture it is the incoming light that orients the membranes, if not in the adult then at least during the growth of the embryonic outer segment. In the absence of light, this idea predicts either an increased curvature of disk membranes or an increased disorientation of outer segments. Light⁹ and electron¹⁴ microscopy studies are inconclusive on this point,

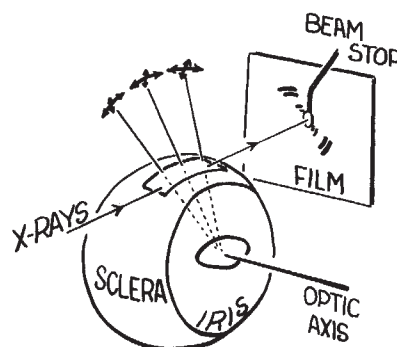


Fig. 2 Diagram of an eye in the X-ray camera. The crossed arrows indicate the asymmetric disorientation of the photoreceptor membranes thought to cause the skew diffraction pattern in Fig. 1*c*. See text.

so it is hoped to test the effects of light deprivation using the technique described in this paper.

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- ¹ Blaurock, A. E., and Wilkins, M. H. F., *Nature*, **223**, 906 (1969).
- ² Gras, W. J., and Worthington, C. R., *Proc. US Nat. Acad. Sci.*, **63**, 233 (1969).
- ³ Davson, J., *The Eye*, 1 (Academic Press, New York and London, 1962).
- ⁴ Guapp, E., in *Anatomie des Frosches* (Vieweg, Berlin, 1899).
- ⁵ Elliott, A. E., *J. Sci. Inst.*, **42**, 312 (1965).
- ⁶ Elliott, G. F., and Worthington, C. R., *J. Ultrastruct. Res.*, **9**, 166 (1963).
- ⁷ Blaurock, A. E., and Wilkins, M. H. F., *Nature* (in the press).
- ⁸ Laties, A. M., Liebman, P. A., and Campbell, C. E. M., *Nature*, **218**, 172 (1968).
- ⁹ Laties, A. M., and Enoch, J. M., *Invest. Ophthalmol.*, **10**, 69 (1971).
- ¹⁰ Schmidt, W. J., *Z. Zellforsch.*, **22**, 485 (1935).
- ¹¹ Clark, A. W., and Branton, D., *Z. Zellforsch.*, **91**, 586 (1968).
- ¹² Stiles, W. S., *Ann. Roy. Coll. Surg.*, **73** (1961).
- ¹³ Young, R. W., *J. Cell Biol.*, **33**, 61 (1967).
- ¹⁴ Eakin, R. M., *J. Cell Biol.*, **25**, Part I, 162 (1965).

Occurrence of Heterozygotes and Homozygotes for the α -Chain Haemoglobin Variant Hb-J(Tongariki) in New Guinea

THE occurrence of the haemoglobin Hb-J(Tongariki) in new locations in the New Guinea area is of interest because this α -chain variant was the first to be found in Melanesian populations. Furthermore, the high proportion of the variant in heterozygotes and the discovery of three apparently homozygous subjects with Hb-J(Tongariki) as the sole major haemoglobin are relevant to current discussion concerning the possible duplication of the human α -chain gene locus.

The haemoglobin variant Hb-J(Tongariki) was discovered¹ in 1967 in Tongariki, an island in the New Hebrides, and shown to have an α -chain substitution ($\alpha 115 \text{ Ala} \rightarrow \text{Asp}$). An electrophoretically similar variant was later shown² to occur in a village in New Britain and its identity with the Tongariki variant established. Two apparently homozygous subjects with only Hb-J(Tongariki) as the major component and no normal adult haemoglobin (Hb-A) were found in the New Britain location, which is separated from the New Hebrides by nearly 2,000 km; both are regarded as having Melanesian populations.

We now report the occurrence of what is almost certainly the same variant in two locations in the New Guinea area. The variant was found at relatively high frequency in five out of seven villages studied on the island of Kar Kar, situated about 15 km from the New Guinea mainland. Three apparently homozygous subjects were found, together with seventy-one heterozygotes, in residents of the Kaul village complex. The variant haemoglobin was not detected in any blood samples from several locations in the Central Highlands of the mainland, but two heterozygotes were found in samples taken from a mainland coastal area opposite Kar Kar island. The known distribution of the variant is summarized in Table 1.

The haemolysates of the three homozygotes from Kaul contained Hb-J as the sole major component with Hb-J₂ (Tongariki) as the only minor component; Hb-A and Hb-A₂

Table 1 Occurrence of Hb-J(Tongariki) on Kar Kar Island and the New Guinea Mainland

Location	No. of subjects	No. with Hb-(A+J)	No. with Hb-J only
Kar Kar island			
Kaul village (initial survey)	648	53	1
Kaul village (selected families)	69	18	2
Six other villages	261	14	0
New Guinea mainland			
Madang coastal region (two villages)	81	2	0
Central Highlands villages about 1,000		0	0

were completely absent (Fig. 1). The globin from Hb-J was fractionated on CM cellulose⁴ and shown to contain normal β -chains and variant α -chains but no normal α -chains. The α -chain chymotryptic peptide $\alpha(110-122)$ ¹, which is part of the tryptic peptide $\alpha\text{Tp XII}$ (100-127), was isolated⁵ and shown to contain one residue of aspartic acid and a deficit of one residue of alanine, as required for Hb-J(Tongariki)¹. A sequence analysis of the $\alpha(110-122)$ peptide to confirm that the substitution is in fact at $\alpha 115$ is in progress, but until this is available it is assumed that the New Guinea variant is Hb-J(Tongariki).

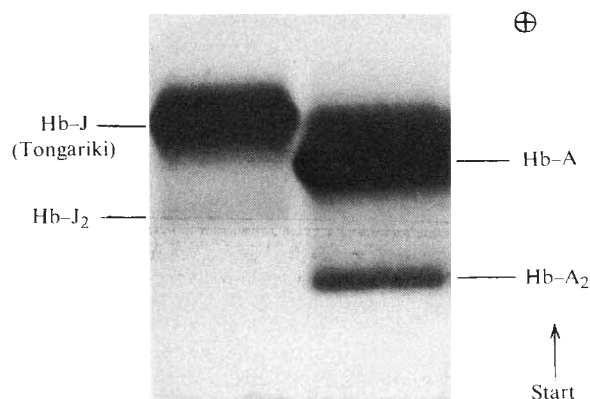


Fig. 1 Starch gel electrophoretic analysis of Hb-J(Tongariki) homozygote and normal adult haemolysates (Tris-EDTA-borate buffer system, pH 8.6; benzidine stain).

The proportion of the variant in the heterozygous subjects was consistently high, with a mean value of 48%, as determined by quantitative starch gel electrophoresis^{6,7}. This high proportion is in agreement with the earlier findings for Hb-J(Tongariki)^{1,2} and in contrast with the lower proportions, about 20-30%, found in many α -chain variants^{8,9}.

The Hb-A₂ levels in the subjects heterozygous for Hb-J(Tongariki) were lower than normal (mean value 1.9%) because of the presence of Hb-J₂(Tongariki) as a second minor proportion. Foetal haemoglobin (Hb-F) was not detected in the heterozygotes or homozygotes by electrophoretic analysis in agar gel at pH 6.2 (refs. 10, 11).

The starch gel electrophoretic analyses of all the samples were inspected for any indication of elevated Hb-A₂ that might indicate the presence of the trait condition for β -thalassaemia. Out of more than 2,000 samples, about ninety were selected on this basis for quantitation of their Hb-A₂ levels, together with a large number of controls, and twenty-seven were found to be at or above the upper normal limit of 3.5%. No haemoglobin H (Hb- β_4), haemoglobin Barts (Hb- γ_4) or other abnormal haemoglobins were seen.

The examination of blood films from twenty-three subjects on Kar Kar island, selected to include several heterozygotes and one homozygote for Hb-J(Tongariki) and several with elevated Hb-A₂, revealed four with definite red cell abnormalities. These included two with raised Hb-A₂ and one with electrophoretically normal haemoglobins. The finding of some subjects with raised

Hb-A₂ levels suggests the presence of the β -thalassaemia gene at low frequency in the population, but the presence of other thalassaemia genes, not causing either an elevation of the Hb-A₂ level or the appearance of Hb-F, has not been excluded. The absence of Hb-H in more than 2,000 samples indicates that α -thalassaemia must be relatively rare. The failure to find any stigmata of thalassaemia in the blood films of six heterozygotes and a homozygote for Hb-J(Tongariki) makes it unlikely that these subjects are also carriers of either α - or β -thalassaemia in the trait form.

The observed frequency of heterozygosity for Hb-J(Tongariki) corresponds to an apparent α^J (Tongariki) gene frequency of about 0.04 for the largest group of random samples (Kaul). As noted by Abramson *et al.*³, it is difficult to be certain for such small endogamous communities that the samples are truly random, but it is evident that, as in the New Britain group, the α^J (Tongariki) gene frequency is appreciable. An analysis of the complex family relationships of the subjects studied on Kar Kar is still in progress, but it has already been shown that for at least one of the three homozygotes for Hb-J(Tongariki) both parents are heterozygotes and that there is no evidence for thalassaemia in this family group.

The high incidence of the heterozygous state for Hb-J(Tongariki) on Kar Kar island and in the two previously studied locations, its occurrence at low frequency on the coastal mainland and its apparent absence in the Central Highlands, suggest that the α^J (Tongariki) gene may prove to be a useful genetic marker for the study of population migration in Melanesia. The regional variations in the incidence of the α^J gene, in relation to several other serum and red cell polymorphisms, will be reported in detail elsewhere.

There has recently been considerable discussion concerning the possible duplication of the human α -chain gene. One argument which has been advanced⁸ in support of this hypothesis is that, unless thalassaemia is also present, α -chain variants usually occur in heterozygotes as a much lower proportion (up to 20%) of the total haemoglobin than β -chain variants (up to 50%), the unstable haemoglobins being excluded from the comparison. The lower proportion of an α -chain variant in the heterozygous state could then be accounted for on the basis that its synthesis is directed by a single mutant gene of the two pairs of genes, normally coding for identical α -chains, which have arisen by α -chain gene duplication. Although many α -chain variants are known which occur at these lower proportions, the collected data⁹ show that for α -chain variants generally the range is essentially continuous, from less than 10% up to 50% of the total haemoglobin, with 20–30% as the most frequent proportion. Hb-J(Tongariki) is thus another example of a "high-level" α -chain variant, like Hb-G(Honolulu) and Hb-G(Philadelphia)⁹. It has also been suggested⁸ that duplication of the α -chain locus could account for the notable variability and genetic complexity of α -thalassaemic states. In support of the hypothesis a Hungarian family has been described¹² in which there is unequivocal evidence for two major α -chain loci.

In the case of Hb-J(Tongariki), however, the complete absence of components containing normal α -chains in the homozygotes, and the consistently high proportion of the variant in the heterozygotes, together with the absence of any evidence for α -thalassaemia, provide further support for the conclusion³ that, at least in some populations carrying the Hb-J(Tongariki) gene, the α -chain locus is not duplicated. As emphasized by Abramson *et al.*³ this conclusion does not preclude the possibility of duplication, either in particular families or in other populations in which the heterozygotes for "low-level" α -chain variants have only about 20% of the variant haemoglobin.

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- ¹ Gajdusek, D. C., Guiart, J., Kirk, R. L., Carrell, R. W., Irvine, D., Kynoch, P. A. M., and Lehmann, H., *J. Med. Genet.*, **4**, 1 (1967).
- ² Booth, P. B., Vines, A. P., and Saave, J. J., *Archeol. Phys. Anthropol. Oceania*, **4**, 115 (1969).
- ³ Abramson, R. K., Rucknagel, D. L., Schreffler, D. C., and Saave, J. J., *Science*, **169**, 194 (1970).
- ⁴ Clegg, J. B., Naughton, M. A., and Weatherall, D. J., *Nature*, **207**, 945 (1965).
- ⁵ Huehns, E. R., in *Chromatographic and Electrophoretic Techniques*, second ed. (edit. by Smith, I.), **2**, 291 (Heinemann, London, 1968).
- ⁶ Gratzer, W. B., and Beaven, G. H., *Clin. Chim. Acta*, **5**, 577 (1960).
- ⁷ Gordon, A. H., in *Laboratory Techniques in Biochemistry and Molecular Biology* (edit. by Work, T. S. and Work, E.), **1**, 123 (North-Holland, Amsterdam and London, 1969).
- ⁸ Lehmann, H., and Carrell, R. W., *Brit. Med. J.*, **4**, 748 (1968).
- ⁹ De Jong, W. W., *Structural characterization of some mutants of human haemoglobin; including two new variants*, thesis, Univ. Leiden (1969).
- ¹⁰ Robinson, A. R., Robson, M., Harrison, A. P., and Zuelzer, W. W., *J. Lab. Clin. Med.*, **50**, 745 (1957).
- ¹¹ Gratzer, W. B., and Beaven, G. H., *J. Chromatog.*, **5**, 315 (1961).
- ¹² Brimhall, R., Hollan, S., Jones, R. T., Koler, R. D., and Szelenyi, J. G., *Clin. Res.*, **18**, 184 (1970).

Multiple Alpha Chain Loci for Human Haemoglobins: Hb J-Buda and Hb G-Pest

It is well established that at least two independent loci control the structure of human haemoglobin A^{1,2}. Although the primary structure of the β -chain is determined by a single genetic locus, there are conflicting reports that the α -chain is controlled by one³ or two^{4,5} loci. We now describe genetic and chemical studies of a Budapest family which possess two abnormal α -chain haemoglobins, Hb J-Buda and Hb G-Pest, and review evidence for multiple loci for the α -chain.

A 48 yr old Hungarian male with erythrocytosis (originally considered to be polycythaemia rubra vera) had an abnormally high haemoglobin concentration, erythrocyte count and haematocrit, but normal values for leucocytes, platelets and pulmonary function tests (full details will be published later). Starch gel electrophoresis⁶ of a haemolysate of his blood revealed a fast major component (Hb J-Buda) migrating like Hb J, a slow major component (Hb G-Pest) migrating like Hb G, as well as normal Hb A (Fig. 1). Blood samples from three of his four sons contained Hb J-Buda in addition to normal Hb A. Blood from the fourth son was electrophoretically normal. Results for other members of this family are illustrated in Fig. 2. In addition to individual II-7, two of his brothers, II-5 and II-10, also have erythrocytosis, and electrophoresis revealed the two abnormal haemoglobins as well as Hb A. So far, six members of the family: I-5, II-1, III-3, III-10, III-11, and III-12, have been found to have Hb J-Buda and Hb A. III-9 and III-15 have Hb G-Pest and Hb A. Erythrocytosis is not present in those who are heterozygous for only Hb J-Buda or Hb G-Pest. Normal members include I-3, II-3, II-8, II-18, II-20, II-22, II-24, III-13, III-16, and III-17. All others are either dead or have not been studied.

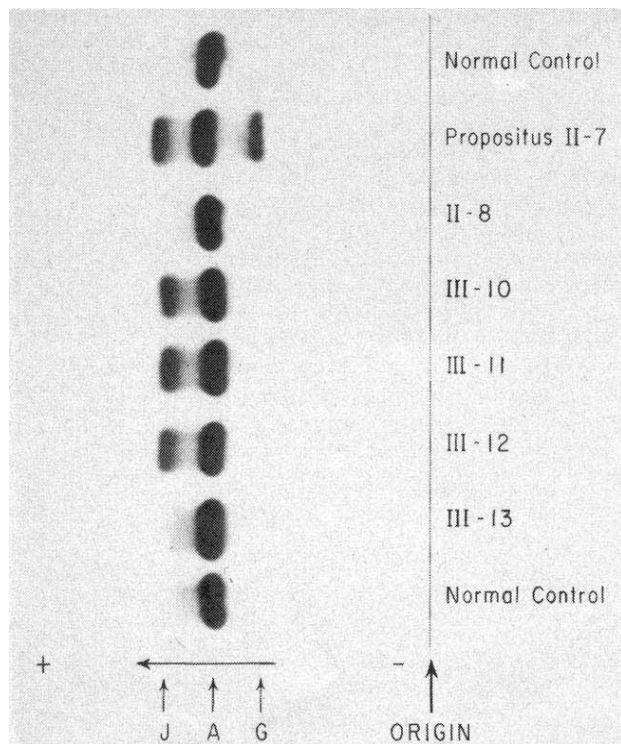


Fig. 1 Starch gel electrophoresis at pH 8.6 of haemoglobins from a normal control, individual II-7, his wife II-8, and four sons III-10, III-11, III-12, and III-13.

When haemoglobins of the three affected brothers (II-5, II-7, II-10) were isolated on columns of 'DEAE-Sephadex', about 2% was identified by starch gel electrophoresis as Hb G₂-Pest and Hb A₂ near the front (Fig. 3). The order of elution the major peaks was Hb G-Pest, Hb A, and Hb J-Buda with relative amounts of 23, 54 and 21%, respectively. The Hb J₂-Buda component was not detected, presumably because it was unresolved from Hb G. Similar results have been observed for three samples of blood from the propositus and

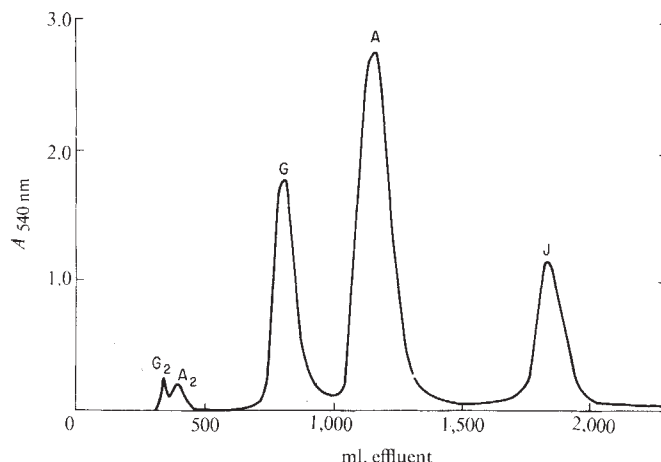
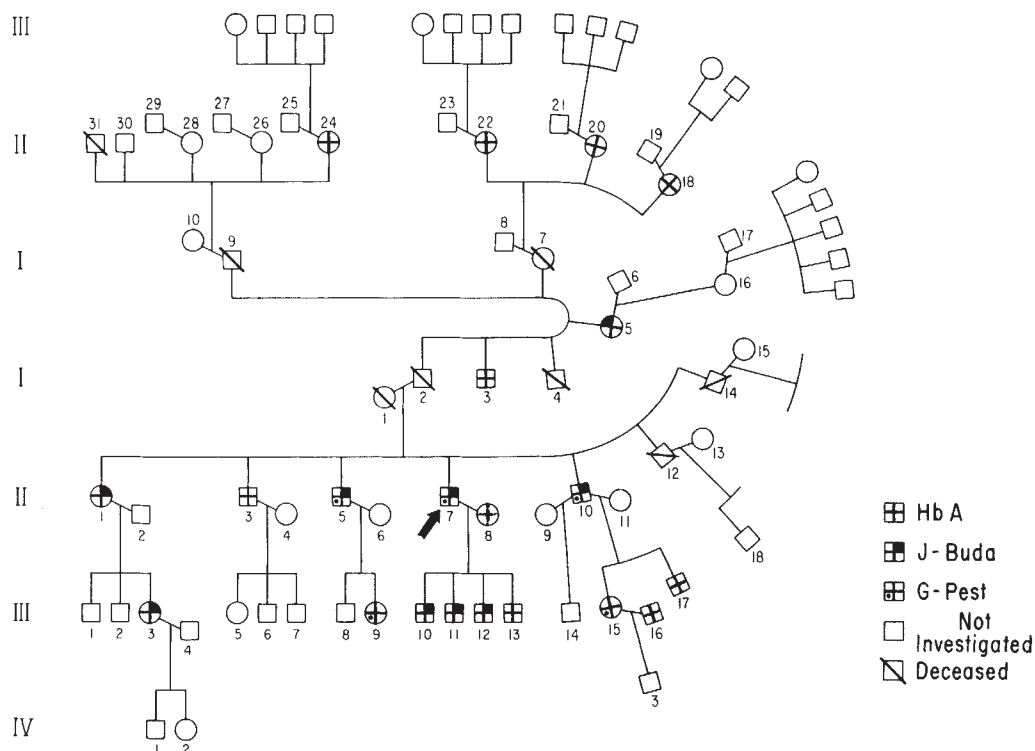


Fig. 3 Chromatogram of haemoglobins from propositus separated on a column of 'DEAE-Sephadex'. Electrophoresis on starch gel indicated the zones to be as marked, G₂, A₂, G, A, and J, respectively.

for single samples from each of his two affected brothers. The relative amounts of haemoglobins determined for individual III-15 were 21% Hb G-Pest and 77% Hb A and from individual III-10 were 16% Hb J-Buda and 81% Hb A.

Fig. 4 shows the results of subunit hybridization⁸ of Hb G-Pest and Hb J-Buda isolated by chromatography. Samples of whole haemolysate from individual II-7 in line eleven and of normal haemolysate in line twelve serve as reference markers. Lines one and two contain mixtures of Hb A and canine Hb before (+) and after (×) hybridization. The anodal product in line two is $\alpha_2^{\text{Canine}}\beta_2^{\text{A}}$ and is similar in mobility to Hb J-Buda; the cathodal product is $\alpha_2^{\text{A}}\beta_2^{\text{Canine}}$ and has the same mobility as Hb C or Hb A₂ (ref. 9). The hybridization of Hb G-Pest with canine Hb (line four) failed to produce $\alpha_2^{\text{A}}\beta_2^{\text{Canine}}$ but instead reveals a slower band near the origin which must contain an abnormal α^{G} chain from Hb G-Pest. Mixtures of Hb J-Buda and canine Hb are shown before and after hybridization in lines five and six. The absence of

Fig. 2 Pedigree of Hungarian family with two abnormal haemoglobins in addition to normal Hb A.



electrophoretically distinguishable products could be due to failure to dissociate and recombine, or to similar electrophoretic mobilities of Hb J-Buda and $\alpha_2\text{Canine}\beta_2^A$ product, and of canine Hb and $\alpha_2\beta_2^{\text{Canine}}$ product. This was tested by hybridizing Hb J-Buda with Hb S as in line eight and with Hb C as in line ten. The band with the mobility of Hb A produced on hybridization with Hb S is consistent with the presence of two electrophoretically similar products, one containing normal α chains from Hb S and normal β chains from Hb J-Buda (that is, Hb A) and the second containing abnormal α' chains from Hb J-Buda and abnormal β^S chains from Hb S (that is, $\alpha_2'\beta_2^S$). This interpretation is supported by the results of hybridization of Hb J-Buda with Hb C (line 10). The second band from the origin is believed to contain the fast α' chains from Hb J-Buda and the slow β^C chains from Hb C. This doubly abnormal hybrid ($\alpha_2'\beta_2^C$) is resolved from the Hb A product which is the third band from the origin in line 10.

The presence of two abnormal α chains in addition to normal α and β chains in the blood of the propositus was confirmed by chromatography using CM-cellulose and an ionic gradient in 8 M urea¹⁰. The results in Fig. 5 reveal normal β chains, fast α' chains, normal α chains, and slow α^G chains. Chain separation of purified haemoglobin zones from Fig. 3 yielded only normal β chains from all three major components, slow α^G chains from Hb G-Pest, fast α' chains in Hb J-Buda, and normal α chains in the Hb A component.

Chemical analysis of the abnormal α chains is in progress and will be reported in detail later. Results are complete for the fast α' chain of Hb J-Buda which has a substitution of an asparaginy residue in place of the normal lysyl residue in position 61. Results are incomplete for the slow α^G chains of Hb G-Pest, but the data are consistent with an amide change in either the ninth or twelfth tryptic peptide.

Major amounts of two abnormal haemoglobins as well as normal adult haemoglobin are rare in the same individual, resulting from unusual states of aggregation, as with Hb Richmond¹¹; from heterozygosity at both α and β chain loci as in the case of combinations of Hb Hopkins II and Hb S^{1,12}, and of Hb G and Hb C¹³; or, as our results demonstrate, from the occurrence of two different α chain mutants among four genes at two loci. Analysis of the pedigree with special reference to the haemoglobins inherited by the children of

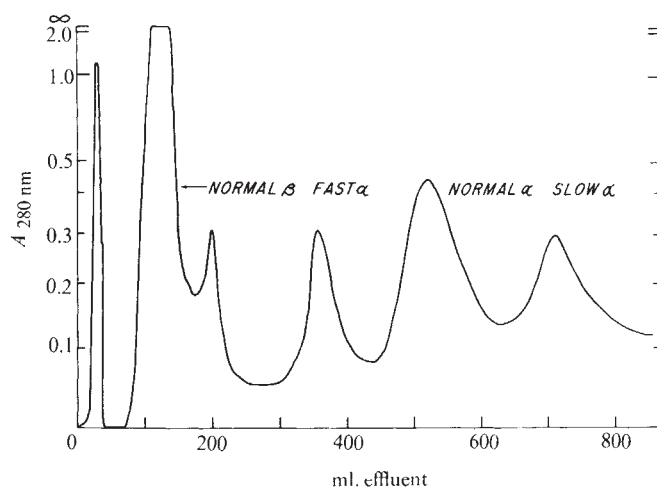


Fig. 5 Chromatographic separation of chains from globin prepared from a haemolysate of blood from II-7.

II-7, and his two affected brothers, II-5 and II-10, indicates that genes coding for the two abnormal α chains are at separate loci. If they were alleles at a single locus, the two normal offspring*, III-13 and III-17, could not have been produced. Moreover, it is possible to test for linkage between the two α -chain loci. In the absence of direct information about I-1 and I-2, the offspring of II-5, II-7, and II-10 are the only informative members of the family. The occurrence of two normal offspring among seven favours independent segregation of mutant and normal alleles at the two loci. Alternatively, if these two resulted from crossing over between two loci on the same chromosome, the recombination fraction of 2/7 argues against close linkage. Therefore, the simplest explanation consistent with all the data in this family is that there are two α -chain loci, that the mutant gene for Hb J-Buda occurs at one locus, that the gene for Hb G-Pest occurs at a second locus, and that the Buda locus and the Pest locus are not closely linked.

There is conflicting evidence for the number of loci controlling the structure of the α chain of haemoglobin^{3,4,15,16}. Many mammals examined have at least two kinds of α -chain¹⁷⁻²² and in some there is evidence for at least two loci^{17,18}. Although genetic studies of α -thalassaemia have been used to argue for two α -chain loci^{23,24}, an analysis of data from Thailand¹⁶ does not discriminate between the one and two locus models. The study of Hb J-Tongariki³ argues for one locus. On the other hand, the results from the Hungarian family are not compatible with a single locus and simple Mendelian inheritance. It is possible, as Abramson *et al.*³ have suggested, that human populations are heterogeneous, some individuals having only one structural locus and others having two. This hypothesis of heterogeneity in the number of α chain loci could account not only for differences found between the Hb J-Tongariki and the Budapest families, but also explain differences in the relative amounts of abnormal α -chain in presumed heterozygotes for the same mutant⁴ and differences in phenotypes among individuals and families with α -thalassaemia.

A second hypothesis is that two α chain loci exist in all human populations but that one of the two loci can be suppressed by genes such as those associated with various kinds of α -thalassaemia. Abramson *et al.*³ considered and excluded the possibility of α -thalassaemia in their family because of the lack of any substantial abnormalities of erythrocyte morphology. If the genetic interpretation of α -thalassaemia described by Koler *et al.*¹⁶, or some modification of it is correct, alleles at one of the two α chain loci could be suppressed without

* Comparison of other genetic markers for III-13 and II-7 and II-8 indicates a 94-95% probability of correct biologic parentage²⁴. Similar studies are being done to exclude false paternity of III-17.

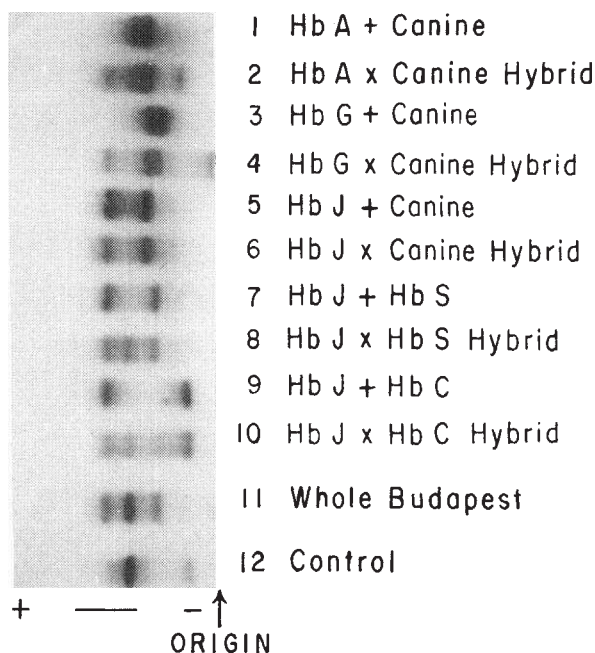


Fig. 4 Starch gel electrophoresis of subunit hybridization tests of Hb G-Pest and Hb J-Buda. See text for detail.

apparent haematological findings unless the other locus is also affected by a mutant allele. Such suppression of one locus could be due to abnormal structure alleles at that locus or to modifying genes. Until haematological, chemical, or genetic methods are developed for the determination of the genotype of individuals who may be carrying the mild α -thalassaemia or similar genes with certainty, it is not possible to decide between these two hypotheses.

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Note added in proof: Preliminary results of studies of genetic markers obtained since submitting this paper for publication raise questions about the biological relationship of individuals III-13 and III-17 to the family. Until results of further marker studies are completed, the conclusions of non-allelism and non-linkage of the genes producing Hb J-Buda and Hb G-Pest must remain in question.

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- ¹ Smith, E. W., and Torbert, J. V., *Bull. Johns Hopkins Hosp.*, **101**, 38 (1958).
- ² Huehns, E. R., and Shooter, E. M., *J. Med. Genet.*, **2**, 48 (1965).
- ³ Abramson, R. K., Rucknagel, D. L., Shreffler, D. C., and Saave, J. J., *Science*, **169**, 194 (1970).
- ⁴ Lehmann, H., and Carrell, R. W., *Brit. Med. J.*, **4**, 748 (1968).
- ⁵ Brimhall, B., Hollan, S., Jones, R. T., Koler, R. D., Stocklen, Z., and Szelenyi, J. G., *Clin. Res.*, **18**, 184 (1970).
- ⁶ Huisman, T. H. J., *Adv. Clin. Chem.*, **6**, 231 (1963).
- ⁷ Huisman, T. H. J., and Dozy, A. M., *J. Chromatog.*, **19**, 160 (1965).
- ⁸ Itano, H. A., Singer, S. J., and Robinson, E., in *Biochemistry of Human Genetics*, Ciba Foundation Symp. (edit. by Wolstenholme, G. E. W., and O'Connor, C. M.), 96 (Churchill, London, 1959).
- ⁹ Huehns, E. R., Shooter, E. M., and Beaven, G. H., *J. Mol. Biol.*, **4**, 323 (1962).
- ¹⁰ Clegg, J. B., Naughton, M. A., and Weatherall, D. J., *J. Mol. Biol.*, **19**, 91 (1966).
- ¹¹ Efremov, G. D., Huisman, T. H. J., Smith, L. L., Wilson, J. B., Kitchens, J. I., Wrightstone, R. N., and Adams, H. R., *J. Biol. Chem.*, **244**, 6105 (1969).
- ¹² Itano, H. A., and Robinson, E. A., *Proc. US Nat. Acad. Sci.*, **46**, 1492 (1960).
- ¹³ Atwater, J., Schwartz, I. R., and Tocantins, L. M., *Blood*, **15**, 901 (1960).
- ¹⁴ Hummel, K., Schmidt, V., and Ihm, P., *Z. Immun. Forsch.*, **137**, 320 (1969).
- ¹⁵ Kattamis, E., and Lehmann, H., *Human Hered.*, **20**, 156 (1970).
- ¹⁶ Koler, R. D., Jones, R. T., Wasi, P., and Pootrukul, S., *Ann. Human Genet.*, **34**, 371 (1971).
- ¹⁷ Adams, H. R., Wrightstone, R. N., Miller, A., and Huisman, T. H. J., *Arch. Biochem. Biophys.*, **132**, 223 (1969).
- ¹⁸ Wade, P., Barnicot, N., and Huehns, E., *Nature*, **215**, 1485 (1967).
- ¹⁹ Wilson, J. B., Wrightstone, R. N., and Huisman, T. H. J., *Nature*, **226**, 354 (1970).
- ²⁰ Oliver, E., and Kitchen, H., *Biochem. Biophys. Res. Commun.*, **31**, 749 (1968).
- ²¹ Ranjekar, P. K., and Barnabas, J., *Comp. Biochem. Physiol.*, **28**, 1395 (1969).
- ²² Hilse, K., and Popp, R. A., *Proc. US Nat. Acad. Sci.*, **61**, 930 (1968).
- ²³ Koler, R. D., and Rigas, D. A., *Ann. Human Genet.*, **25**, 95 (1961).
- ²⁴ Lehmann, H., *Lancet*, ii, 78 (1970).

Lysosomes, pH and the Anti-malarial Action of Chloroquine

MALARIA parasites¹ or mammalian liver² suitably exposed to chloroquine rapidly form autophagic vacuoles; in the case of the parasites, these are visible under the light microscope because they contain clumps of pigment, the remains of haemoglobin digestion (Fig. 1). These striking morphological changes are well known but not correspondingly well understood.

Equally striking, and presumably important in the production of these changes, is the ability of red cells that contain chloroquine-sensitive malaria parasites to concentrate the drug to high levels. The concentration in such cells relative to that in non-infected erythrocytes has been variously estimated at 100:1³, 300-500:1⁴ and 40:1⁵. The mechanism by which this concentration gradient is attained has not been explained satisfactorily, but it has been shown^{3,5,6} that erythrocytes containing chloroquine-resistant parasites accumulate considerably less of the drug than those containing chloroquine-sensitive ones. It is therefore important to explain the concentrating mechanism if chloroquine resistance is to be understood and combatted.

Polet and Barr⁷ showed that the initial uptake of drug is energy-independent, and therefore not due to active transport, though this is followed by a slower, energy-dependent phase. Fitch^{5,6} considers that binding sites in the malarial cell are responsible for the accumulation of the drug. Rollo⁸ suggested that some unspecified change in the general metabolism of the resistant parasites might raise their overall pH, and that this would lead to a reduced accumulation of chloroquine.

The ionization constants of chloroquine (pK_{a1} 10.2, pK_{a2} 8.1)⁹ indicate that at physiological pH 18% of the drug is in the monoprotonated form which is soluble in lipid and hence capable of passing into cell membrane¹⁰. The drug would pass out readily from the membrane phase into a more acid cytoplasm with production of the doubly protonated form, insoluble in lipid and incapable of passing back into the membrane. Chloroquine would thus cross the membrane into the cell provided that the pH inside the cell remained lower than that of the plasma. We consider that Rollo's theory is valid, but it would relate more readily to the lysosomal organelles of a cell, which are considered to be at acid pH¹¹, than to the general cytoplasm. Indeed, chloroquine can be shown by fluorescence microscopy to be concentrated in the lysosomes of mammalian cells¹². The digestive vacuoles of malarial parasites (and other protozoa¹⁴) are considered to be analogous to mammalian secondary lysosomes, and to be at acid pH. No direct evidence for this has been obtained, although one of the haemoglobin-digesting enzymes of the parasite has been shown to have a pH optimum of 4¹³.

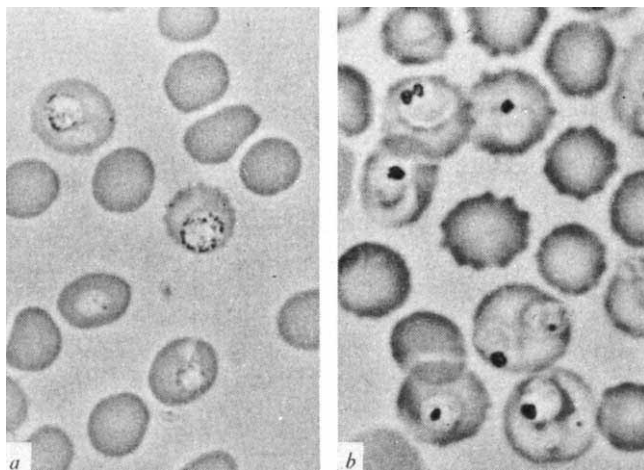


Fig. 1 Normal (a) and clumped (b) pigment of *Plasmodium berghei* N strain.

The protonation of chloroquine within the digestive vacuoles would deplete them of hydrogen ions and uptake of drug would then cease unless more acid were secreted into the vacuoles or new acid-containing vacuoles were formed; either case would require energy, thus explaining the second, energy-dependent phase of chloroquine uptake described by Polet and Barr.

Depleting the food vacuole of acid would change its internal pH from that required for the most efficient action of the digestive enzymes, digestion of haemoglobin would be considerably impaired, and the supply of amino-acids to the parasite dramatically reduced. Because the mature mammalian erythrocyte is virtually impermeable to some amino-acids¹⁵ the normal rapid growth of the parasite would be halted. Starvation may itself lead to the formation of an autophagic vacuole in some organisms^{16,17}, but inhibition of digestive enzymes would itself be a sufficient cause. Inhibition of mammalian lysosomal enzymes with specific antibodies results in the formation of grossly enlarged lysosomes which are considered to be autophagic vacuoles¹⁸ and are similar to those found in congenital storage diseases in which a lysosomal enzyme is lacking¹⁹.

Chloroquine in high concentrations inhibits certain proteolytic enzymes and this may add to its effects on digestive processes of the parasites²⁰. If chloroquine acts by the simple mechanism proposed above, then any treatment which raises the pH inside the digestive vacuole should have the same effects as those caused by chloroquine, and these effects should be visible as "clumping" of the malarial pigment. The internal pHs of even such metabolically active cells as *E. coli*²¹ and Ehrlich ascites tumour²² are very dependent on the pH of the external medium. Whether the pH inside digestive vacuoles is as readily affected is a matter for conjecture, but there is no reason to suppose that it is completely immune to pH changes in the surrounding cytoplasm. It might therefore be expected that simply increasing the pH of the medium in which parasitized erythrocytes were suspended would cause the same morphological effects as exposure to chloroquine. The results of experiments to test this *in vitro* are shown in Table 1.

Animals, their diet and the *Plasmodium berghei* N strain malarial parasites were as described previously²³. The incubation method will be described elsewhere. Briefly, dilute suspensions of parasitized erythrocytes in Medium 199 (Bio-Cult Labs. Ltd) plus 10% foetal calf serum (Flow Laboratories)²⁴ were adjusted to pH 7.4–8.0 with sodium bicarbonate and/or gassing with 95% air/5% CO₂, and incubated in a roller tube apparatus at 37° C for 80 min.

Table 1 shows that raising the extracellular pH correspondingly increases the amount of clumping. Under the light microscope, clumping was identical to that caused by chloroquine. Table 2 shows the effect of cycloheximide on the clumping produced by either an increase in pH or by chloroquine. Both are inhibited, and to about the same extent, indicating that whatever the mechanism of induction of the autophagic vacuoles, the mechanisms of production both involve protein synthesis.

It might be postulated that one of the effects common to chloroquine administration and pH rise is a general inhibition of cell metabolism. Inhibitors of protein synthesis, nucleic acid

Table 2 Antagonism by Cycloheximide of Clumping of Malarial Pigment caused by High pH or by Chloroquine

Treatment		Percentage of pigmented cells completely clumped
pH	Drug	Mean (n=2)
7.4	—	3
7.7	—	23
8.0	—	36
7.4	Chloroquine (10 ⁻⁶ M)	92
7.4	Cycloheximide (10 ⁻⁵ M)	5
7.4	Chloroquine (10 ⁻⁶ M) and cycloheximide (10 ⁻⁵ M)	28
8.0	Cycloheximide (10 ⁻⁵ M)	14

synthesis and respiration, however, do not cause clumping. Indeed, continuing protein synthesis has been shown to be necessary for the production of clumping²⁵, from which it can be inferred that the experimental alterations of cell pH were not drastic enough to affect at least one synthetic system, that synthesizing protein.

Chloroquine has well known effects on membrane stability; at low concentrations (about 10⁻⁴ M) it stabilizes erythrocyte membranes, but at high concentrations (over 10⁻³ M) it labilizes them²⁶. Although the concentration in plasma does not rise above 10⁻⁶ M²⁷, and the red cell membrane is therefore unaffected, it is quite possible that the concentration of chloroquine in the malarial food vacuole could rise sufficiently to labilize its membranes²⁸. The formation of the autophagic vacuole could then be regarded as a defensive attempt by the cell to segregate the hydrolytic enzymes thus released into its cytoplasm. But it has been shown²⁹ that clumping appears before breakdown of RNA can be detected, suggesting that lysis of cellular constituents follows rather than precedes autophagic vacuole formation. Furthermore, raising the pH to 8 did not cause haemolysis of the red cells although clumping occurred. If the actions of chloroquine and of pH change involve similar mechanisms then it is unlikely that the initial action of chloroquine on food vacuoles is due to labilization of their membranes, although this may be a secondary effect which releases high concentrations of drug into the parasite cytoplasm.

The simplest possibility is that chloroquine acts initially by raising the pH of the malarial food vacuoles, thereby reducing the digestion of haemoglobin by the parasite and preventing its growth. Moreover, sporogonic and exoerythrocytic stages of the parasites, which do not possess digestive vacuoles, are unaffected by chloroquine. Chloroquine-resistant erythrocytic parasites concentrate less chloroquine than sensitive ones^{3,5,6} and apparently degrade less haemoglobin as they produce less pigment³⁰. These observations could be simply explained as a reduction of the acidity of the food vacuoles of resistant parasites. Lack of acid (the high affinity binding sites of Fitch) would reduce the uptake of chloroquine, and also prevent effective haemoglobin digestion by enzymes requiring an acid pH for maximum activity. Even at a relatively high pH, however, some enzyme activity would persist, resulting in the production of the small amounts of pigment which are seen in chloroquine-resistant strains. The inability of the resistant parasite to digest sufficient haemoglobin would necessitate an alternative source of amino-acids, possibly by transamination of citric acid cycle intermediates³¹.

Chloroquine must act in the same way in other biological systems although the effects will not be so readily visible as in the malarial parasite. It must raise the pH of mammalian lysosomes, which concentrate this and many other basic drugs³². This leads to the interesting speculation that in many cases the effect of drugs on intralysosomal pH may be the same as that of chloroquine, and that this may be an integral part of their action.

This work was supported in part by the MRC and the WHO, and in part by a grant from the US Army Medical Research and Development Command, Department of the Army.

Table 1 Effect of Raised pH and of Chloroquine on Pigment of Malarial Parasites *in vitro*

pH	Treatment	Percentage of pigmented cells in which pigment was completely clumped
	Drug	Mean ± s.e. (No. of experiments)
7.4	—	4.7 ± 1.5 (6)
7.8–8.0	—	54.3 ± 6.0 (8)
7.4	Chloroquine 10 ⁻⁶ M	85.7 ± 4.6 (6)

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- ¹ Warhurst, D. C., and Hockley, D. J., *Nature*, **214**, 935 (1967).
- ² Abraham, R., Hendy, R., and Grasso, P., *Exp. Mol. Pathol.*, **9**, 212 (1968).
- ³ Macomber, P. B., O'Brien, R. L., and Hahn, F. E., *Science*, **152**, 1374 (1966).
- ⁴ Polet, H., and Barr, C. F., *J. Pharmac. Exp. Ther.*, **164**, 380 (1968).
- ⁵ Fitch, C. D., *Proc. US Nat. Acad. Sci.*, **64**, 1181 (1969).
- ⁶ Fitch, C. D., *Science*, **169**, 289 (1970).
- ⁷ Polet, H., and Barr, C. F., *J. Pharmac. Exp. Ther.*, **168**, 187 (1969).
- ⁸ Rollo, I. M., *Fed. Proc.*, **27**, 537, *Abstr.* (1968).
- ⁹ Irvin, J. L., and Irvin, E. M., *J. Amer. Chem. Soc.*, **69**, 1091 (1947).
- ¹⁰ Albert, A., *Selective Toxicity*, fourth ed., 458 (Methuen, London, 1968).
- ¹¹ De Duve, C., and Wattiaux, R., *Ann. Rev. Physiol.*, **28**, 435 (1966).
- ¹² Allison, A. C., and Young, M. R., *Life Sci.*, **3**, 1407 (1964).
- ¹³ Cook, L., Grant, P. T., and Kermack, W. O., *Exp. Parasitol.*, **11**, 372 (1961).
- ¹⁴ Kudo, R. R., *Protozoology*, fifth ed., 123 (Thomas, Springfield, Illinois, 1966).
- ¹⁵ Winter, C. G., and Christensen, H. N., *J. Biol. Chem.*, **239**, 872 (1964).
- ¹⁶ Malkoff, D. B., and Buetow, D. E., *Exp. Cell Res.*, **35**, 58 (1964).
- ¹⁷ Levy, A., and Elliott, A. M., *J. Protozool.*, **15**, 208 (1968).
- ¹⁸ Tulkens, P., Trouet, A., and Van Hoof, F., *Nature*, **228**, 1282 (1970).
- ¹⁹ Hers, H. G., and Van Hoof, F., in *Lysosomes in Biology and Pathology* (edit. by Dingle, J. T., and Fell, H. B.), **2**, 19 (North-Holland, Amsterdam, 1969).
- ²⁰ Cowey, F. K., and Whitehouse, M. W., *Biochem. Pharmacol.*, **15**, 1071 (1966).
- ²¹ Kashkett, E. R., and Wong, P. T. S., *Biochim. Biophys. Acta*, **193**, 212 (1969).
- ²² Poole, D. T., Butler, T. C., and Waddell, W. J., *J. Nat. Cancer Inst.*, **32**, 939 (1964).
- ²³ Peters, W., *Exp. Parasitol.*, **17**, 80 (1965).
- ²⁴ Trigg, P. L., *Parasitology*, **59**, 925 (1969).
- ²⁵ Warhurst, D. C., and Robinson, B. L., *Trans. Roy. Soc. Trop. Med. Hyg.*, **65**, 11 (*abstract*) (1971).
- ²⁶ Inglot, A. D., and Wolna, E., *Biochem. Pharmacol.*, **17**, 269 (1968).
- ²⁷ Berliner, R. W., Earle, D. P., Taggart, J. V., Zubrod, C. G., Welch, W. J., Conan, N. J., Bauman, E., Sudder, S. T., and Shannon, J. A., *J. Clin. Invest.*, **27**, 98 (1948).
- ²⁸ Allison, A. C., in *The Interaction of Drugs and Subcellular Components* (edit. by Campbell, P. N.), 218 (Churchill, London, 1968).
- ²⁹ Warhurst, D. C., and Williamson, J., *Chem.-Biol. Interactions*, **2**, 89 (1970).
- ³⁰ Peters, W., Fletcher, K. A., and Staubli, W., *Ann. Trop. Med. Parasitol.*, **59**, 126 (1965).
- ³¹ Howells, R. E., Peters, W., Homewood, C. A., and Warhurst, D. C., *Nature*, **228**, 625 (1970).
- ³² Allison, A. C., and Young, M. R., *Life Sci.*, **3**, 12 (1964).

Temperature-sensitive Mutants of Adenovirus defective in Interferon Induction at Non-permissive Temperature

SEVERAL different types of human adenoviruses induce interferon in chick embryo cells^{1,2} and it has been proposed that the penton antigen of the virus is responsible for this¹. The evidence supporting this hypothesis, however, depends chiefly on sensitivity to trypsin, and in our opinion the adenovirus functions responsible for inducing interferon have not been clearly identified. We have isolated temperature-sensitive

(*ts*) mutants of adenovirus type 5³, which provided us with a possible system for identifying these functions. With this view in mind, we have tested ten *ts* mutants, classified into nine complementation groups⁴, for their ability to induce interferon in chick embryo cells at both the permissive (31° C) and non-permissive (38° C) temperatures. This brief communication presents evidence that two of these mutants, which complement each other in their *ts* functions, do not induce interferon at the non-permissive temperature, but induce it at the permissive temperature.

To produce interferon, primary chick embryo fibroblasts (CEF) seeded 2-3 days earlier were infected with wild type or mutant virus at an input multiplicity of 20 plaque-forming units (p.f.u.)/cell. After 2 h for adsorption at 38° C, the cultures were washed once with Eagle's medium and then overlaid with Eagle's medium+2% calf serum. Half were then incubated for 3 days at 38° C and the other half for 4 days at 31° C. At these times, the culture fluids were harvested, centrifuged for 10 min at 3,000 r.p.m. to remove cell debris, and heated at 56° C for 30 min to inactivate residual virus. Interferon was assayed by measuring the inhibition of plaque formation by vaccinia virus on CEF cells. For titration, serial two-fold dilutions of the culture media from the CEF were prepared and 2 ml. aliquots of the dilutions were added to CEF monolayers on 50 mm plastic Petri dishes. After overnight incubation (12-18 h) the aliquots were withdrawn, cultures were washed, challenged with 80-100 p.f.u. of vaccinia virus/dish, and plaques were counted 2 days later. The interferon titre is expressed as the PDD 50—the reciprocal of the dilution causing a 50% depression of the control plaque count.

The results of two representative experiments involving induction of interferon at 31° C and 38° C using wild type and ten *ts* mutants of adenovirus type 5 are shown in Table 1. In both experiments, all mutants and the wild type virus induced good yields of interferon in CEF cells at 31° C. At 38° C, the wild type and all *ts* mutants induced normal yields of interferon, except *ts*18 and *ts*19 which failed completely to induce interferon at 38° C. In these experiments we have adsorbed virus at 38° C before further incubation at 31° C or 38° C. As normal yields of interferon were attained in cultures infected with *ts*18 and *ts*19 and incubated at 31° C, it is most unlikely that the temperature-sensitive defect is expressed at the stage of virus adsorption to the cells. Corroborative evidence stems from the fact that virus adsorbed at 31° C for 2 h is still unable to induce interferon in CEF subsequently incubated at 38° C, while inducing normal levels when incubated at 31° C. The possibility that penetration or uncoating (or both these events) of the mutant viruses is thermosensitive in CEF cells is not yet ruled out and experiments are in progress to determine if this is the case.

We have reported efficient complementation⁴ in HeLa cells between *ts*18 and *ts*19, and this suggests that these mutations are located in different genes which probably control different viral functions. To determine if *ts*18 and *ts*19 also complement

Table 1 Induction of Interferon at 31° C and 38° C by Wild Type and *ts* Mutants of Adenovirus Type 5

Virus	Interferon titre *			
	Experiment 1		Experiment 2	
	31° C	38° C	31° C	38° C
5 wild type	128	128	256	128
5 <i>ts</i> 1	256	128	256	128
5 <i>ts</i> 2	256	32	128	128
5 <i>ts</i> 3	256	128	128	64
5 <i>ts</i> 4	128	64	256	64
5 <i>ts</i> 13	256	128	128	64
5 <i>ts</i> 14	256	128	128	64
5 <i>ts</i> 17	256	128	128	64
5 <i>ts</i> 18	128	<4†	64	<4
5 <i>ts</i> 19	64	<4†	64	4
5 <i>ts</i> 20	128	128	128	64

* PDD 50.

† These results were confirmed on at least five separate occasions.

each other in CEF cells with respect to interferon induction, double infection of CEF cells with 10 p.f.u./cell of each mutant at 38° C was carried out. The results were negative, as no interferon was induced in doubly infected cells in conditions where wild type virus induced normal levels of interferon. We are continuing to investigate this failure to complement, for we cannot yet rule out the possibility that an insufficient amount of virus penetrated CEF cells in order to ensure double infection of most cells.

Further investigations involving temperature shift experiments may allow us to pinpoint the time after initiation of infection at which the mutants function to induce interferon. The physiology of the various mutants is being examined, in both permissive HeLa cells and in the non-permissive CEF cells. The combined genetic and biochemical approach should eventually permit us to define the viral function(s) involved in interferon and to identify the viral component(s) or product(s) responsible.

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¹ Béládi, I., and Pusztai, R., *Z. Naturforsch.*, **22b**, 165 (1967).

² Ho, M., and Kohler, K., *Archiv. für die Gesamte Virusforschung*, **22**, 69 (1967).

³ Williams, J. F., Gharpure, M., Ustacelebi, S., and McDonald, S., *J. Gen. Virol.*, **11**, 95 (1971).

⁴ Williams, J. F., and Ustacelebi, S., *J. Gen. Virol.* (in the press).

Membrane Reorganization during Secretion in *Tetrahymena*

DURING our investigation of the membrane systems of the ciliated protozoan *Tetrahymena pyriformis*, we have discovered a unique differentiation within the plasma membrane that corresponds to sites of discharging mucocysts, and we have been able to reconstruct a representation of the process of membrane fusion at the moment of discharge.

Earlier electron microscope studies of *Tetrahymena*¹⁻³ have shown that the surface of these ciliates is a highly differentiated pellicle consisting of ridges, grooves and underlying fibres. The outer layer of this pellicle is a unit membrane that is continuous over the entire body. Directly below the unit membrane is a system of flat membrane-bound vesicles, the alveoli, which are interrupted at regular intervals by cilia and mucocysts. The cilia which cover *Tetrahymena* are neatly ordered in rows, which run from the anterior to the posterior end of the cell, and are known as the primary meridians; three to five mucocysts are found in between adjacent cilia along the primary meridians. Along the secondary meridians which are located between the primary meridians, there are complete rows of mucocysts but no cilia.

The mucocyst is an elongated sac-like structure, surrounded by its own unit membrane. In thin sectioned fixed material, the undischarged mucocyst contains an electron opaque crystalline material with a longitudinal periodicity of about 180 Å. The mucocyst is reported to develop from the endoplasmic reticulum³. During this process the immature mucocyst enlarges, and the material inside condenses and finally crystallizes. Concurrently, the mucocyst moves from the interior of the cell to end up resting near the plasma membrane until discharge. By morphogenetic, descriptive and physiological criteria, the mucocyst is a characteristic merocrine secretion granule of this cell.

When the mucocyst discharges, its membrane fuses with the cell membrane. Then the mucocyst enlarges and the membrane

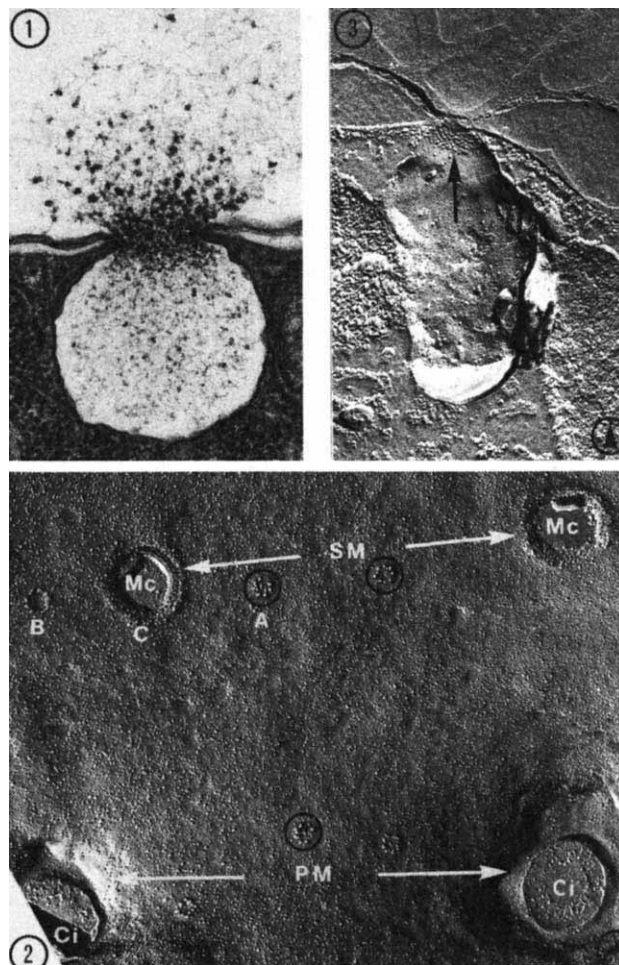


Fig. 1 Discharging mucocyst showing completed fusion of mucocyst and cell membranes ($\times 43,200$).

Fig. 2 Freeze-fracture of face A of the cell membrane showing part of a primary meridian (PM) demarcated by cilia (Ci), and a secondary meridian (SM). Rosettes (encircled) indicate sites where mucocysts will discharge. Discharged mucocysts also are present (MC). The membrane appearance changes sequentially from A to B to C as the discharge occurs ($\times 43,200$). Encircled black arrowheads show shadowing direction.

Fig. 3 Freeze-fracture of a mucocyst (face A) just before fusion with the cell membrane. Note the particle array at the tip of the mucocyst (arrow) ($\times 43,200$).

momentarily ruptures at the point of juncture (Fig. 1). Simultaneously, the material within the mucocyst loses its crystalline appearance, expands, and is expelled from the cell and it apparently dissolves to form a slime coat around the organism⁴.

We fixed *Tetrahymena* from 20 h old cultures (mid-log phase) with 0.5% glutaraldehyde in 50 mM phosphate buffer, pH 6.9, transferred them to 20% glycerol in the same buffer for 2 h or overnight, froze them in 'Freon 22' at -150°C and stored them at liquid nitrogen temperature. Frozen specimens were fractured in a Balzers apparatus at -115°C usually without etching. The fractured surface was replicated with platinum and carbon and prepared for electron microscopy.

Many fractures pass through the plasma membrane along a unique plane to reveal internal membrane structure⁵. As is usual, two different appearances of the membrane can be discerned: one, face A, a view towards the cytoplasm, and the complementary view, face B, towards the exterior. The primary meridians are demarcated by the cilia that are easily recognized in cross-fracture (Fig. 2). On face A, at sites corresponding to undischarged mucocysts along both primary and secondary meridians, there are rosettes about 600 Å in

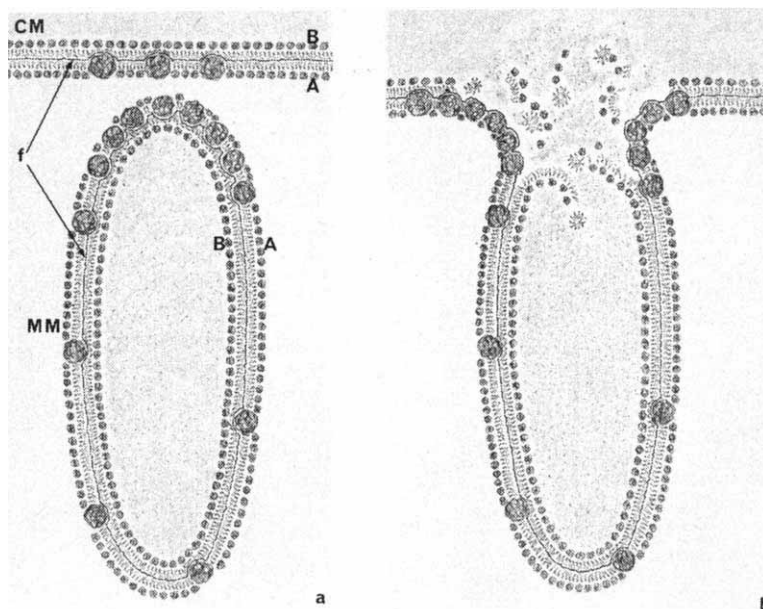


Fig. 4 Diagram of membrane fusion model. *a*, Undischarged mucocyst, just prior to fusion of cell (CM) and mucocyst (MM) membranes. Dark line (f) indicates fracture plane separating *A* and *B* faces. *b*, Discharging mucocyst showing reorganization of the membranes.

diameter consisting of 8–11 150 Å diameter particles. Sometimes there is an additional particle in the centre of the rosette. The distribution of the rosettes is somewhat irregular and varies from specimen to specimen. There are more mucocysts along a meridian than rosettes. This suggests that the arrangement of particles into a rosette is transient, forming only when the mucocyst approaches within a critical distance of the surface membrane.

Discharging mucocysts are also present in fractures of the plasma membrane. They appear as circular pockets of varying depth with diameters ranging from 600 Å to 2100 Å. At the bottom of the pocket, the fracture reveals a smooth membrane face. Between the discharging mucocysts intact rosettes can still be seen, which indicates that discharge takes place asynchronously. As seen in the fracture plane (face *A*), the sequence of membrane fusion of an individual mucocyst and a single specific rosette (site) in the plasma membrane is as follows (Fig. 2). First, a depression appears within the rosette. Then the octagon of particles expands and the pocket enlarges and deepens. The particles of the rosette become separated at the pocket's edge and additional smaller particles can be seen.

One face *A* fracture of a mucocyst membrane approaching the plasma membrane is shown in Fig. 3. At the anterior end of this organelle we have found a formation of closely packed particles about 100 Å in diameter. This particle array is absent in mucocysts located further from the point of fusion, and is therefore considered a rearrangement of the mucocyst membrane that occurs just before fusion, matching the rearrangement of the plasma membrane to form the rosette. We can account for the additional particles that are found in fractures of discharging mucocysts, if face *A* of the mucocyst membrane fuses with face *A* of the cell membrane (Fig. 4).

The resemblance of the apposed particle arrays of mucocyst and cell membranes to the arrays within cell junctions⁶ is suggestive. We propose that, as the mucocyst reaches the surface, protein intercalations within the membranes suddenly rearrange themselves into particles to open a continuous hydrophilic channel (an electrotonic junction)⁷ from the exterior of the cell to the inside of the mucocyst. The concentrated material within the organelle might then produce a gradient so that water would rush in osmotically through these newly formed channels to swell the organelle and provide the explosive force of secretion.

Rearrangements of the *B* faces of the two membranes must occur during this process. One possibility is that some of the micelles comprising face *B* at the point of fusion disperse in

the aqueous mixture. Fracture faces within the pocket have a smooth appearance and are not down deep. In this region, the fracture may pass either through the mucocyst content or through a mucocyst *B* face that is undergoing transformation.

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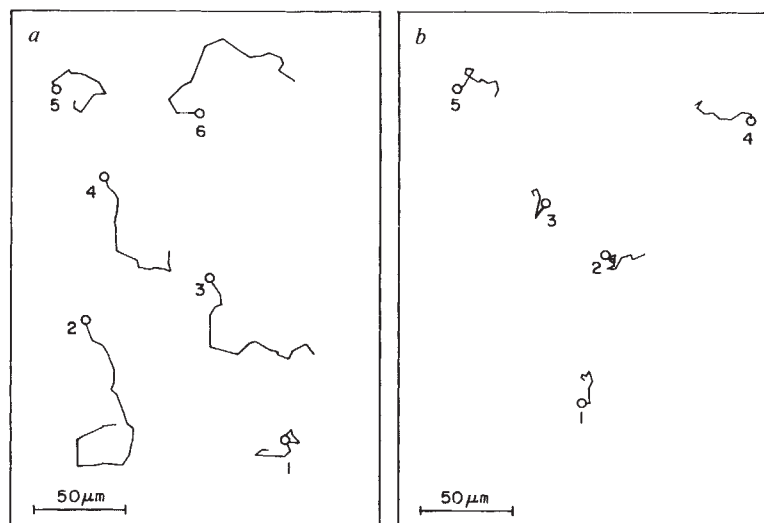
- ¹ Pitelka, D. R., *J. Protozool.*, **8**, 75 (1961).
- ² Allen, R. D., *J. Protozool.*, **14**, 553 (1967).
- ³ Tokuyasu, K., and Scherbaum, O. H., *J. Cell Biol.*, **27**, 67 (1965).
- ⁴ Pitelka, D. R., *Electron-Microscopic Structure of Protozoa*, 56 (Macmillan, New York, 1963).
- ⁵ Branton, D., *Proc. US Nat. Acad. Sci.*, **55**, 1048 (1966).
- ⁶ Gilula, N. B., Branton, D., and Satir, P., *Proc. US Nat. Acad. Sci.*, **67**, 213 (1970).
- ⁷ Payton, B. W., Bennett, M. V. L., and Pappas, G. D., *Science*, **166**, 1641 (1969).

Regulation of Cell Motility by Cyclic AMP

FIBROBLASTS exhibit a locomotory behaviour in cultures on a plane surface. An undulating membrane or leading lamella forms at the frontal edge and may be the locomotory organ of the cell. When the leading lamellae of two cells collide, several events occur. The forward motion of the cells is stopped, the cells contract, the undulations of the membrane cease, and movement proceeds in a different direction. This has been termed contact inhibition of movement¹. When cells become confluent, movement is considerably decreased, presumably due to contact on all surfaces, and at confluency there are restrictions on cell growth which are apparently related to the population density of the cells. This has been termed contact or density-dependent inhibition of growth^{2–4}. At present, no strong evidence connects contact inhibition of movement and growth, but as transformation often disturbs both properties a connexion between them may exist^{3,5}.

How these contact phenomena are regulated is not clear, although the interaction of the two surfaces may generate a

Fig. 1 *a*, Migration of untreated L-929 fibroblasts over 150 min. The analysis was begun about 20 min after addition of fresh medium. The migration rate ranged from 0.3 $\mu\text{m}/\text{min}$ to 1 $\mu\text{m}/\text{min}$. Individual cell paths have been arbitrarily oriented to avoid overlapping lines. *b*, Migration of L-929 fibroblasts treated with 1.2 mM dbc-AMP over 150 min. Migration analysis was begun about 20 min after addition of fresh medium containing the dbc-AMP. The migration rate was 0.2 $\mu\text{m}/\text{min}$ to 0.25 $\mu\text{m}/\text{min}$.



signal which regulates these processes⁵. As adenosine-3',5'-cyclic monophosphate (cyclic AMP) is known to be an important regulatory molecule in many systems^{6,7}, and as the enzyme adenyl cyclase is present in the plasma membrane of cells, the involvement of cyclic AMP in contact inhibition of growth has been suggested⁸⁻¹².

We have reported that endogenous levels of cyclic AMP rise three to four-fold in 3T3 cells at confluency¹³ and, if this elevation of the cyclic AMP concentration is important in the regulation of the contact inhibition of movement, addition of N⁶,O^{2'}-dibutyryl cyclic AMP (dbc-AMP) or prostaglandin E₁ (PGE₁), which elevates cyclic AMP levels¹⁴ by activating adenyl cyclase¹⁵, should decrease motility in sub-confluent cultures of fibroblasts.

In this study, we have used time lapse cinematography to analyse the effects of dbc-AMP and PGE₁ on the motility of L-929 cells and found that cyclic AMP can regulate the motility of fibroblasts.

L-929 fibroblasts¹⁶ were grown in Eagle's minimal essential medium (MEM) with 10% foetal calf serum¹³ (Flow Laboratories, Rockville, Maryland) in modified multipurpose tissue culture chambers¹⁷⁻¹⁹ and the medium was changed daily. Cultures were incubated at 36°C in a time-lapse photographic apparatus (EMDECO-Brinkmann, Great Neck, New York) and photographed by Cinn II cameras by a split-frame photographic technique (unpublished) at $\times 25$ magnification. Photographs were taken at 1 min intervals starting about 3 h after planting the cells. Cell migration was analysed by projecting the film at $\times 550$ magnification and tracing the movement of each cell. Cells were randomly selected for analysis, except that the few cells which entered mitosis or migrated out of the field of vision during the time period were excluded. PGE₁ (a gift from Dr John Pike, Upjohn Co., Kalamazoo, Michigan) was diluted from a 10 mg/ml. (ethanol) stock solution to 50 $\mu\text{g}/\text{ml}$. with MEM immediately before use.

L-929 cells exist in many morphological forms²⁰. Within one culture, cells in both a rounded and an extended configuration are observed. Moreover, the cells constantly change from one form to the other. Cells in both forms show considerable movement across the substratum²⁰ (Fig. 1). The locomotory organ of the retracted cells appears to be an undulating membrane which develops at various locations on the cell periphery in the direction of cell movement. Undulating membranes are observed at the leading edge of extended cells and also seem to be involved in cell locomotion. Furthermore, movement is caused by a shift of cell mass during withdrawal and re-extension of the cellular processes.

By 20 min after the addition of dbc-AMP, the movement of the cells is dramatically decreased (Fig. 1). (For technical reasons, satisfactory photographs cannot be obtained immediately after the new medium containing dbc-AMP is added.)

Undulating membranes are still apparent but the frequency of undulations is reduced. The motion of cells in both the retracted and extended morphological forms is affected.

After 4-5 h of incubation with dbc-AMP, the cellular processes become greatly extended so that the cells are considerably elongated⁹. The motion of the cells in this extended form remains inhibited. With continued incubation in the presence of dbc-AMP, the cells continue to divide but at a decreased rate.

After 4 days incubation with dbc-AMP, it was removed. Very shortly after the removal, cells become as active as the untreated cells (Fig. 2). Within 20 min, numerous cells had both retracted their extended processes and begun to move about rapidly. A few cells retained the extended, bipolar form and their motility was hindered. However, within 1-2 h these also withdrew their processes and immediately began to move rapidly (Fig. 3).

PGE₁ activates adenyl cyclase in L-929 cell homogenates¹⁵ and elevates intracellular levels of cyclic AMP in intact cells¹⁴.

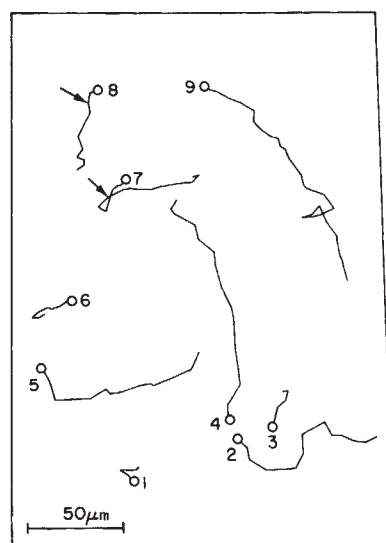
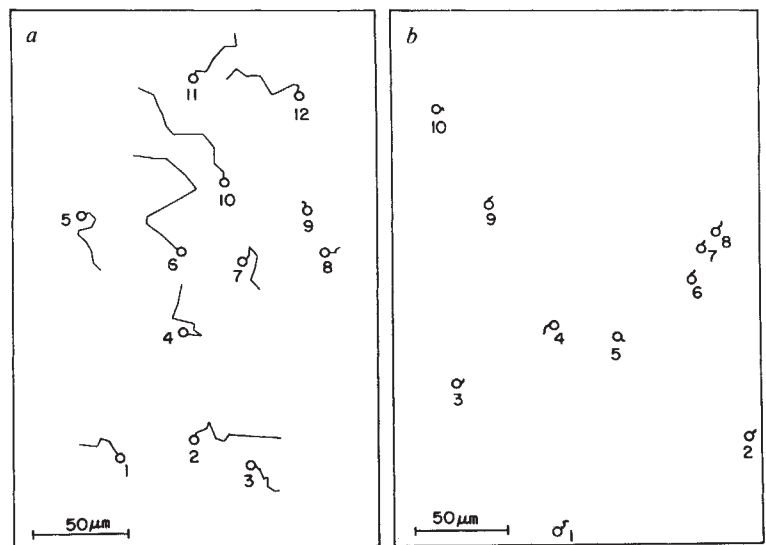


Fig. 2 Migration of L-929 fibroblasts over 150 min beginning about 20 min after removal of dbc-AMP. Cells had been treated with dbc-AMP for 4 days. Cells 2, 4, 5 and 9 were in the rounded state throughout the 150 min. Their migration was about 0.8 to 1 $\mu\text{m}/\text{min}$. Cells 1 and 6 remained in the extended bipolar form. Their migrations were about 0.15 to 0.2 $\mu\text{m}/\text{min}$. Cells 7 and 8 were in the extended form at the beginning, but at the arrows they rapidly retracted the processes and began rapid movement. Cell 7 changed shape after 60 min, cell 8 after 72 min. Cell 3 was a rounded cell similar to cells 2, 4, 5 and 9, but with a very small undulating membrane.

Fig. 3 Migration of L-929 fibroblasts over 90 min. Migration analysis was begun 20 min after addition of fresh medium (a) or fresh medium containing PGE₁ (50 µg/ml.) (b). The migration rate was less than 0.1 µm/min in the PGE₁-treated cells.



Incubation of the cells with PGE₁ would be expected, therefore, to inhibit cell locomotion as well. Very shortly after addition of PGE₁, the motion of the cells is also hindered (Fig. 3).

In this study we have shown that dbc-AMP or PGE₁ decreases the motility of L-929 cells in sub-confluent cultures. When dbc-AMP is removed, the motility rapidly returns to normal. Therefore, we propose that cyclic AMP mediates contact inhibition of movement in sub-confluent cultures. In confluent cultures of 3T3 cells, movement and also growth are inhibited³. Associated with this contact inhibition of movement and growth is a rise in cyclic AMP levels¹³. In many lines which fail to demonstrate contact inhibition of movement and growth, cyclic AMP levels do not rise¹³. Therefore, it seems very likely that cyclic AMP mediates contact inhibition of movement and growth in confluent normal cells.

Various earlier experiments suggested that contact inhibition of movement and growth are controlled by a common regulatory mechanism. Cells which show inhibition of movement at contact tend to grow into monolayers, whereas transformed cells which have lost the contact phenomena easily migrate over one another and grow into multiple layers¹⁵. In a contact inhibited line, cell motility and division cease at confluency, but when a wound is made into the confluent culture, cells rapidly begin to migrate into the wound, synthesize DNA, and divide until the wound area is filled²¹⁻²³. Addition of fresh serum to confluent cultures stimulates both cell movement and cell division, and the increase in motility and DNA synthesis are proportional to the amount of fresh serum added²⁴.

In studies with many lines of transformed fibroblasts, the cells treated with dbc-AMP grow more slowly^{8,9,11,12}. In only a few cases, however, is the saturation density decreased (ref. 11 and unpublished work of G. S. J. and I. P.). Thus, although cyclic AMP probably participates in contact inhibition of growth, factors other than cyclic AMP are probably also involved in mediating the process and are defective in transformed cells. Cyclic AMP may mediate both contact inhibition of growth and motility in normal cells, but in some transformed cells one or both processes may escape from control by cyclic AMP or the cells may have lost the capacity to control their cyclic AMP levels.

The rapid action of dbc-AMP and PGE₁ on motility suggests that their control of motility is independent of any action on RNA or protein synthesis. If, as has been proposed, microfilaments regulate the motility of cells^{25,26}, then cyclic AMP may modify the microfilament proteins, perhaps by stimulating its phosphorylation.

Cyclic AMP promotes motility of cells as diverse as sperm²⁷ and *E. coli*²⁸. In sperm, its mechanism of action is unknown, but in *E. coli* it is required for the synthesis of flagellae. Once

flagellae are made, the cells remain motile even with a fall in cyclic AMP levels.

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- ¹ Abercrombie, M., and Heaysman, J. E. M., *Exp. Cell Res.*, **6**, 293 (1954).
- ² Golde, A., *Virology*, **16**, 9 (1962).
- ³ Todaro, G. J., Green, H., and Goldberg, B. D., *Proc. US Nat. Acad. Sci.*, **51**, 66 (1964).
- ⁴ Stoker, M. G. P., and Rubin, H., *Nature*, **215**, 171 (1967).
- ⁵ Abercrombie, M., *In Vitro*, **6**, 129 (1970).
- ⁶ Pastan, I., and Perlman, R. L., *Nature New Biology*, **229**, 5 (1971).
- ⁷ Robison, G. A., Butcher, R. W., and Sutherland, E. W., *Ann. Rev. Biochem.*, **37**, 149 (1968).
- ⁸ Bürk, R. R., *Nature*, **219**, 1272 (1968).
- ⁹ Johnson, G. S., Friedman, R. M., and Pastan, I., *Proc. US Nat. Acad. Sci.*, **68**, 425 (1971).
- ¹⁰ Hsie, A., and Puck, T. T., *Proc. US Nat. Acad. Sci.*, **68**, 358 (1971).
- ¹¹ Sheppard, J. R., *Proc. US Nat. Acad. Sci.*, **68**, 1316 (1971).
- ¹² Ryan, W. L., and Heidrick, M. L., *Science*, **162**, 1484 (1968).
- ¹³ Otten, J., Johnson, G. S., and Pastan, I., *Biochem. Biophys. Res. Commun.* (in the press).
- ¹⁴ Pastan, I., Johnson, G. S., Otten, J., Peery, C. V., and d'Armiento, M., *Fed. Proc.*, **30**, 1047 (1971).
- ¹⁵ Peery, C. V., Johnson, G. S., and Pastan, I., *J. Biol. Chem.* (in the press).
- ¹⁶ Sanford, K. K., Earle, W. R., and Likely, G. D., *J. Nat. Cancer Inst.*, **9**, 229 (1948).
- ¹⁷ Morgan, W. D., and Dawe, C. J., *J. Nat. Cancer Inst.*, **26**, 133 (1961).
- ¹⁸ Morgan, W. D., *In Vitro*, **2**, 126 (1966).
- ¹⁹ Rose, G. G., *Texas Rep. Biol. Med.*, **12**, 1074 (1954).
- ²⁰ McQuilkin, W. T., and Earle, W. R., *J. Nat. Cancer Inst.*, **28**, 763 (1962).
- ²¹ Todaro, G. S., Lazar, G., and Green, H., *J. Cell Comp. Physiol.*, **66**, 325 (1965).
- ²² Gurney, T., *Proc. US Nat. Acad. Sci.*, **62**, 906 (1969).
- ²³ Dulbecco, R., and Stoker, M. G. P., *Proc. US Nat. Acad. Sci.*, **66**, 204 (1970).
- ²⁴ Baker, J. B., and Humphreys, T., *Proc. US Nat. Acad. Sci.* (in the press).
- ²⁵ Carter, S. B., *Nature*, **213**, 261 (1967).
- ²⁶ Spooner, B. S., Yamada, K. M., and Wessells, N. K., *J. Cell Biol.*, **49**, 595 (1971).
- ²⁷ Garbers, D. L., Lust, W. D., First, N. L., and Lardy, H. A., *Biochem.*, **10**, 1825 (1971).
- ²⁸ Yokota, T., and Gots, J. S., *J. Bact.*, **103**, 513 (1970).

BOOK REVIEWS

Science and Humanity

Public Affairs. By C. P. SNOW. Pp. 224. (Macmillan: London, 1971.) £3.00.

LORD SNOW has become more pessimistic. Reconsidering his own essays he notes that the "mood of 1968 and 1970 has altered, not grossly but perceptibly, from anything written ten years before". There are objective reasons. The prospect of overpopulation is becoming inescapably graphic. Though the likelihood of thermonuclear war is receding somewhat (the world is, politically, a more complicated, wary place than it was during the period of outright American-Russian confrontation), the gap between the rich and poor societies is widening. Contrary to more sanguine estimates made only ten years ago, many underdeveloped countries are growing relatively poorer each decade, and some are becoming absolutely poorer. If current projections hold, the average income of a United States citizen will, by the end of the century, be forty times that of his Central or South American neighbour. "It is hard to imagine such a world," says Snow, but not some of the social and political consequences which will ensue.

Snow's lucid sadness arises from an observation which has been central to his personality and work as a writer and civil servant. He is acutely vulnerable to the barriers which seem to separate intelligence from action. Why is it that individuals and societies are unable to act in time in accord with their own rational insights? What stubborn void falls between the springs of the human intellect and those of collective behaviour? The population crisis, the dilemma of industrial pollution, the follies of nationalism or ideological passion have been clearly diagnosed. Snow himself has, since the late 1930s, been among the watchmen at the gate, giving early warning. Yet foreknowledge "has made no effect, and of course it could have made no effect unless the tide was flowing with us. The tide has continued to flow in the opposite direction".

Why? The question is, obviously, as old as the Greek tragic poets, with their unsparing vision of man as a creature walking open-eyed into disaster. But its urgency for Snow stems from the fact that he cannot accept any of the traditionally available religious or mythical answers. Notions such as the theological depravity of man, Koestler's arguments for a neuro-physiological

discrepancy between neo-cortex and atavistic drives, the conservative image of the species as unalterably ruled by greed, stupidity and unreason, are equally unacceptable to Snow. He is a man committed to an axiom of sanity, to a belief in the progressive energies of human rationality. Even where his novels touch on themes of self-destruction, on absurdities and irrational compulsions, a strong current of sobriety, of braced good sense, prevails.

The triumphs of intellect, moreover, the great architectures of artistic vision and philosophic order, are not enough. This is the crux of Snow's isolation from the literary-academic world and the underlying cause of the attacks he has experienced. Snow is in no way an anti-intellectual: his sensibility catches fire when he writes of the achievements of a Dirac or a G. H. Hardy. But he deeply distrusts the sheltered purities of the mandarin mind; he is obsessed with the prevalence in the academic and the "intelligentzia" of knowledge without wisdom, of cleverness without empirical ballast. In the final analysis, he puts action first. He regards his writings as "a form of action". He who can does, said Goethe. "All prescripts for action have to be simple. Action itself is simple or it doesn't happen." But if this is so, why the mess in which mankind finds itself, why the incapacity to translate foresight into remedy?

Though conceived from very different points of attack and occasion, the lectures and essays gathered in this book all revert to this vital perplexity. They cover the period from 1959 to 1971, years during which Snow achieved a wide measure of international recognition, re-entered public life and completed his eleven-volume series of novels, *Strangers and Brothers*. All these texts have been previously published, and a number of them, such as the Rede Lecture on "The Two Cultures" or the Godkin Lectures on "Science and Government" given at Harvard in 1960, are very well known. Nevertheless, they do make for a continuous, self-qualifying statement and it is good to have them in one volume.

The "two cultures" debate has been conducted so widely and vehemently—a secondary literature of polemic and exegesis has sprung up around Snow's initial pronouncement—that one hesitates to add anything further. It is plain that Snow had touched on a vital nerve of discomfort, and that much of what he said about "literary semi-literacy" and the obscurantist politics of

certain twentieth-century literary masters demanded close attention. I would make only two marginal points. The bitter quarrel with Dr Leavis went much beyond the immediate issues: it seems to me to involve two sharply contrasting views of what England and English life ought to be like under the stress of American technology. Second, it may be that Snow's use of the word "cultures" led to misunderstanding. Had he, for example, said "orientations", "psychological stance", his case might have been given a far more responsive hearing. That the orientation of the scientist toward the future differs sharply from the humanist's inevitable involvement in the past is, surely, undeniable and the polarities and tensions which follow are indeed of the utmost importance if we are to grasp the issues which physics and biology now pose. If anything, the current debates over ecology and the often violent indictments of science and technology urged by certain scientists underline Snow's warning that failures of communication between science and society are dangerous. Where he may have gone wrong is in presenting too uniform a picture of the scientific stance. Scientists are themselves almost as divided in outlook and counsel as are humanists. We are dealing less with a "culture" neatly divided than with numerous models of what a real culture ought to be like at a time when no single human mind or even aggregate of minds seems able to absorb, let alone reorganize, the necessary mass of information.

Snow's narrative of the Tizard-Lindemann quarrel in "Science and Government" retains its fascination. Here the skill of the novelist conjoins perfectly with the experience of the civil servant. Again, the issues which concern Snow go beyond the clash of extraordinary personalities and the tangled interplay of human weakness and strength in vital matters of state. If Tizard "had been able to submit the bombing controversy to the Fellows of the Royal Society, or the general population of professional scientists, Lindemann would not have lasted a week". Yet, in fact, he won the duel and all but exiled Tizard from public life. Once more we find the large gap between rational understanding, between scientific dispassion (all of which were on Tizard's side) and the course of events. The very point of the story is to inquire whether "we can arrange to make choices a little more reasonably", whether truth can be brought to bear

more directly on the conduct of affairs. Snow's conclusion is at once bleak and urgent: do not give extensive and secret powers to any one scientist among non-scientists, and wherever a scientist must carry major political or social weight, look to his human, general qualities no less than to his expertise.

Public Affairs shows Snow's most attractive characteristics: his seriousness, his readiness to reconsider a previous argument and to criticize his own positions, the open, almost boyish awe he still registers before what is truly first class in modern science. Mainly these papers demonstrate courtesy of heart. This happens to be just now, particularly in the world of literature, a rare commodity. GEORGE STEINER

Chemical Evolution

Molecular Evolution: Chemical Evolution and the Origin of Life. Edited by R. Buve and C. Ponnampuruma. (Proceedings of the International Conference on the Origin of Life.) Pp. xi + 560. (North-Holland: Amsterdam and London, 1971.) Dfl. 80; \$22.25.

Prebiotic and Biochemical Evolution. Edited by A. P. Kimball and J. Oro. Pp. x + 296. (North-Holland: Amsterdam and London, 1971.) £7.69.

THE first volume is a compilation of the papers given at a conference on the general subject of the origin of life. By a curious oversight, nowhere in the volume is the place (Pont-à-Mousson, France) or date (April 1970) of the conference given. However, the organizers of the conference (also the editors of this book) planned it as a successor to the chemical evolution meetings that were held in Moscow in 1957 and at Wakulla Springs, Florida, in 1963.

The book has the usual disadvantages of a compilation of this sort. No effort has been made to separate the trivial, the rambling, and the incomplete from the important, the concise, and the thorough. The difficulties in separating wheat from chaff are increased by a simple lack of proof reading (and editing). The reader can only sigh when confronting such prose as "Organized systems created more highly ordered systems and there (sic) high ordered systems finally gained a new quality" (p. 78), and "After collecting the shocked and quenched gas sample, the bulb was detached from the tube . . . and the walls washed . . ." (p. 116). Apparently the authors didn't proof read their own galleys. One author's name is misspelled, in his own article, in two different ways (pp. 78 and 79), and the book displays a liberal sprinkling of typographical errors.

When one reads a volume of this sort, one regrets the common practice of publishing all the papers presented

at a meeting. It ought to be possible to select out the best papers from a conference and include only those in a smaller volume. Perhaps less relevant, less complete, and also-published-elsewhere papers might be included only in abstract form. However the publisher might feel, and however it might damage some of the editors' friendships, it would save the money of the purchaser and the time of the reader.

Having registered some complaints, I should now like to proceed to details that are, in the main, praiseworthy. The book is divided into eight sections. The first, entitled "Introductory Session," contains two papers—the first consisting of some general remarks on the problem of the origin of life by "the father of the field of chemical evolution," A. I. Oparin, and the second a review by M. Florkin of the chemical-evolutionary relevance of organic molecules found in fossils. The second section, which has a "catch-all" title of "General and Theoretical Problems," contains six papers that discuss such subjects as the thermodynamics of the spatial organizations necessary for life, the energetics of the production (both today and on the prebiotic Earth) of biologically relevant compounds, and the origin of optical activity. I liked the discussion on energetics by Morowitz, but found the other five papers short on both newness and clarity.

The third and fourth sections are entitled "Syntheses of Small Molecules" and "Oligomers and Polymers." These sections (approximately 200 pages) are the book's most pertinent ones with respect to the subject matter usually brought to mind by the phrase "chemical evolution." The third section reviews recent progress in studies of the appearance of "biomonomers" (amino-acids, sugars, nucleic-acid bases and so on) under the effects, to take one example, of shock waves, which simulate meteorite impacts on the prebiotic Earth. Every paper in this section is well worth reading. The fourth section is a fine review of the large problem of figuring out how the biomonomers were coupled on the prebiotic Earth to form the proteins, nucleic acids, polysaccharides, and fats. Particularly interesting is the paper by Rich, who suggests that polyesters might "be used as an information-containing polymer in biological systems, although not necessarily those of terrestrial origin". The problems of the origin, accumulation, and utilization of energy-rich polyphosphate compounds are well outlined in six papers by LePort, Schwartz, Ponnampuruma, Halmann, Mathews, Paecht-Horowitz, and their coworkers. Fox and Dose and their colleagues contributed interesting research results on the effects of reacting proteinooids with nucleic acids and of the altered catalytic properties of the pro-

teinooids when they are prepared in the presence of haemin.

The fifth section is entitled "Photochemical Processes," and consists of five papers, all from the Bakh and photosynthesis institutes in Moscow. The authors present data and discussions on such subjects as oxide-enhancement of the rate of porphyrin appearance in a formaldehyde-pyrrole mixture, the effect of pH on the evolution of the photosynthetic apparatus, the appearance of flavins and photoreceptors, and the likely order of appearance of the photosynthetic "System I" and "System II".

The sixth section is ambitiously titled "Origin of Biological Structures." Kaplan presents a most interesting discussion of the chances that "one molecule with a sequence functional for a given enzyme could be expected in 2 g random protein." Other papers in this section review progress on the Oparin coacervate idea and its ramifications, including membrane formations. A paper by Gabel, which really should have been back in the third section with the other polyphosphate papers, emphasizes the widespread occurrence of polyphosphates in contemporary living matter.

The seventh section, "Primitive Biochemistry and Biology," is mostly outside of the area of chemical evolution. It is really a summary of evolutionary biochemical developments that occurred after the first appearance of living cells. Particularly interesting to me, however, were papers by Lipmann on polypeptide syntheses without nucleic acids, by Dayhoff on the amino-acid sequences in diverse organisms, and by Antonov on the variability of DNA base-pairs as a function of the organism's complexity.

The final section is on exobiology. Here the emphasis is on the organic compounds in interstellar space, in meteorites, on planetary surfaces, and on the Moon—the last two subjects were particularly well discussed in papers by Eglinton and Biemann.

For all its unevenness, this volume can be given at least the minimum recommendation the publisher hopes to hear—it belongs to the library of every research group that has an interest in chemical evolution.

Most of the defects of this book, and some additional ones, also apply to the papers on *Prebiotic and Biochemical Evolution* collected from symposia at Houston, Texas, in 1968 and Madrid in 1969. Of the twenty-three papers presented, six had already appeared in the scientific literature, two (those by Krosnovsky and by Rabinowitz *et al.*) are earlier versions of those that appeared in the earlier volume, and other papers have little or nothing to do with biochemical evolution. In four papers (dealing with polypeptic syntheses, nucleic-acid biosyntheses, and ribonuclease structure) the evolutionary motif is com-

pletely ignored, and one wonders why they were included in this volume. To make a long complaint short, I believe that only about half the papers herein presented really belong in a volume of this sort.

The section on "Primitive Biochemistry and Biology," which I thought somewhat out of place in the book on *Molecular Evolution*, would have fitted the subject matter of this volume perfectly. Particularly because both volumes are from the same publisher, I am disappointed that the editors did not get together and split up the material into a "chemical evolution" book and a "biochemical evolution" book.

Regardless of whether they were published elsewhere, the papers in this book that I particularly liked, and whose enumeration will give the flavour of this book, are: (1) The discussion of Britten and Kohne on DNA associations. They show that if "1% of the base pairs are not complementary, the temperature at which dissociation occurs (melting temperature) is about 1° C lower than that for perfectly complementary strands." (2) Jukes's exposition of protein amino-acid sequences as a reflexion of the base sequences in DNA. (3) The summary by McReynolds *et al.* on the formation of nucleic acid-like polymers on the ultraviolet irradiation of deoxyribonucleotides. (4) Fox's summary of the case for the possible route to primitive heterotrophs through the heating of dry amino-acids, followed by solution of the resultant "proteinoid" in warm water, and the appearance of "microspheres" on cooling the solution. (5) The paper by Degens and Matheja that demonstrates enhanced peptide bond formation, in aqueous solutions at 80° C, by the presence of the catalytic surfaces of clay minerals.

I doubt that this volume is worth the purchase price to an individual scientist; but this, too, belongs in the library of any laboratory organization that has an interest in the subject.

RICHARD M. LEMMON

Group Theory

Electronic Energy Bands in Solids. By L. Pincherle. Pp. vi+196. (Macdonald: London, September 1971.) £4.

Group Theory in Solid State Physics. By Hans Waldemar Streitwolf. Pp. 248. (Macdonald: London, September 1971.) £5.

THE theory of electronic energy bands continues to form the basis for much of solid state physics, and consequently it is a subject of great importance to both theoretical and experimental physicists. Professor Pincherle's book will be welcomed as a very valuable addition to the literature on this topic. It provides for postgraduate students a

very clear and thorough introduction to the subject, and leads them to the stage where they should be able to follow the more specialized accounts of contemporary developments. It keeps an excellent balance between the formal exposition of the theory and simple applications, with brief discussions of a number of topics that are related to band theory. The level of presentation is such that it could be read with ease by any graduate in physics or engineering.

In his first two chapters Professor Pincherle has carefully and lucidly presented the basic concepts of band theory, the arguments being illustrated by many well chosen diagrams. The third chapter deals with some aspects of the formalism of band theory, such as momentum Eigen functions and Wannier functions, while the fourth gives an excellent account of the dynamics of electrons in crystals. Although the fifth chapter is entitled "Band Calculation", Professor Pincherle has devoted most of his attention to the construction of Fermi surfaces in the free and nearly-free electron approximations, with relatively little space being given to *ab initio* band calculations. This is a wise choice, as there are already several specialized expositions of this topic. Professor Pincherle has also prudently refrained from attempting to list and describe all the specific calculations that have been carried out. The final chapter deals with the classification of bands using group theory. A knowledge of the fundamental concepts of group theory has been assumed, and then a brief but clear sketch has been given of the sort of results that this theory can predict. Of course nothing more detailed or ambitious is possible in a book pitched at this level, for a complete treatment requires a book to itself.

One such treatment has been given by Dr Streitwolf. His book was first published in 1967 with the title *Gruppentheorie in der Festkörperphysik*. The English edition has been translated by Dr J. B. Sykes, whose translation reads very easily.

The title suggests a much wider range of topics than that which actually appears in the contents, for, as the author admits in his preface, there are a number of applications of group theory in solid state physics which are not mentioned. In particular there is no treatment of magnetic groups, of crystal field theory, and the role of permutation groups in many-electron problems. In fact the book is devoted almost entirely to a study of the crystallographic space groups and their application in the one-electron band theory and the theory of lattice vibrations. It gives a detailed and clear account of these topics. The treatment

is self-contained, and assumes only an elementary knowledge of quantum mechanics and matrix theory, the theory of groups and their representations and the role that they play in quantum mechanics being developed from scratch.

The book has been written for mathematical physicists, and experimentalists may have some difficulties with the presentation. Dr Streitwolf has chosen to develop the subject by deriving first the general (but rather complicated) results on the representations of non-symmorphic space groups, and then, very briefly, treating symmorphic space groups as a special case. This approach has all the advantages of conciseness, but possibly many students would be happier with the alternative development (available elsewhere) in which they can approach the subject in a more gentle fashion, mastering the relatively straightforward theory of symmorphic space groups before tackling the general theory.

Dr Streitwolf has included an account of the theory of double groups, of time-reversal symmetry, and a particularly good description of selection rules. The very important diamond structure has been used throughout as an example to illustrate the various aspects of the theory.

One surprising point for a book written at this level is the sparseness of the list of references. The classic papers in the development of the subject all appear, but there is a serious lack of references to the tabulations of the characters of the 240 space groups. These have actually all been calculated, and there exist at least two books, by Kovalev and by Miller and Love, giving completely comprehensive sets of tables.

J. F. CORNWELL

Oligochaetes

Aquatic Oligochaeta of the World. By R. O. Brinkhurst and B. G. M. Jamieson. With contributions by D. G. Cook, D. V. Anderson and J. van der Land. Pp. xi+860. (Oliver and Boyd: Edinburgh, November 1971.) £12.

MORE than forty years ago Stephenson (1930, *The Oligochaeta*) was of the opinion that the Oligochaeta had become too large for a monograph by any one person. His prediction has so far proved correct, for in this new book no less than five authors have collaborated to produce a long needed, comprehensive work on the aquatic species alone.

The work is divided into two main parts, Biology (Chapters 1-4) and Systematics (Chapters 5-15), each chapter being followed by a list of references. The first part opens with a chapter on anatomy by D. G. Cook and B. G. M. Jamieson and this is followed by a lucid account of the embryology

by D. V. Anderson. The third chapter, distribution and ecology, is in many ways the most useful in the first part of the book. R. O. Brinkhurst provides an excellent summary of the habitat, pollution biology and respiratory physiology of the microdriles and discusses the interactions and dynamics of species in microdrile communities. B. G. M. Jamieson completes Chapter 3 with an account of the distribution of the Alluroideidae and Glossoscolecidae and speculates on the latter's dispersal routes. These two authors share in the final chapter of the first part of the book, on phylogeny and classification, in which they admit that the phylogeny of this group without a fossil history can be advanced only with hesitation and assert that the classification which they propose is phenetic. The classification contains many novel features although fundamentally it has some resemblance with the system proposed by Stolte (1968, *Oligochaeta* [3], *Bronns Kl. Ord. Tierreichs*) which is, incidentally, not cited among the references.

In the second and larger part of the book, keys and diagnoses are provided to the families and most genera and species of the world's aquatic Oligochaeta, the family Enchytraeidae and tribe Glossoscolecini being dealt with only at generic level. Many species are figured and the illustrations are gathered together at the end of each chapter so that comparisons can be made easily. The responsibilities for the eleven chapters are as follows: D. G. Cook—Lumbricidae and Dorydrilidae; R. O. Brinkhurst—Haplotaxidae, Naididae, Tubificidae, Phreodrilidae, Opistocystidae and Enchytraeidae; J. van der Land—Aelosomatidae; B. G. M. Jamieson—Alluroideidae and Glossoscolecidae. (The family Branchiobdellidae is omitted as the authors now exclude it from the Oligochaeta.) R. O. Brinkhurst was responsible for the arrangement and format of the synonymies and scientific index. In the former, the layout and typography are such that the reader cannot easily discover the valid name of a species or where one section ends and another begins, and in the latter, the generic names following specific epithets are needlessly set in parenthesis as if they have only subgeneric status. (Abstracters should note that the names of the new species described in the text are not specially listed either in the index or together elsewhere.) It is regrettable that the publishers permitted a uniformity of type-face and setting for the scientific names, whether used as headings or in synonymies, to mar an otherwise excellent work. Generally, however, all concerned with the work are to be congratulated for filling the need admirably for information on these small oligochaetes to be drawn together in a single volume. R. W. SIMS

Immunology

Journal of Immunological Methods. Edited by F. Borek, J. R. Battisto and E. J. Holborrow, Vol. 1, No. 1. Pp. 106. (North-Holland: Amsterdam and London. September 1971.) One volume of four issues/year, Dfl. 81.00.

Now is boom time for immunologists. There is a flood of new workers and a host of new journals. Old timers groan at the brashness of the late arrivals and creak with the effort of keeping up with the wave of fresh information. Nothing, however, can stem the tide. The latest addition to the literature array is the *Journal of Immunological Methods*. It claims to be unique in its devotion to methods and techniques in immunology, and indeed five of the eight papers in the first issue give accounts of new or relatively new methods. The journal will accept short communications, review-type articles on methods and full-length articles describing original work. If *JIM*, as it will doubtless come to be known, maintains the initial high standard of presentation and content it will fulfil a useful purpose in an area where methods *per se* are broadcast in a haphazard manner through many and diverse journals. It might be helpful if the editors deliberately recruited details of older but much used techniques from their originators or principal users in order that we should always know where to look for technical details of immunological methods. A. J. S. DAVIES

Specific Immunities

Cell Interactions and Receptor Antibodies in Immune Responses. (Proceedings of the Third Sigrd Juselius Symposium.) By O. Makela, Anne Cross and T. U. Kosunen. Pp. xx+472. (Academic: New York and London, July 1971.) £7; \$21.

THIS is a book mainly about lymphocytes with many excellent papers summarizing knowledge about the classification, characteristics and interactions of "T" and "B" cells. There are separate sections on the handling of antigens by factors other than lymphoid cells, the classes of lymphocytes, the reactions of lymphocytes to antigenic stimulation, effector mechanisms following lymphocyte activation as well as the specific topics of cell interactions and the nature of the antigen receptors on "T" and "B" cells. Lymphocyte receptor sites are quite strong antigens and it is now relatively easy to raise antisera against cell bound immunoglobulin receptors of "B" cells of mice and similar antibodies have now been raised against human "B" cells. Such antisera are capable of blocking the receptor to its specific

antigen and perhaps the key to antibody specificity will be found by studying the biochemical similarities between an antigen and the antibody which is capable of blocking the specific lymphocyte receptor. The application of such antibodies to the study of receptor sites has indeed led to some surprising new discoveries. For example it has recently been suggested that these sites "float" in the cell surface membrane and in the presence of antigen congregate at one pole before disappearing into the cell, there presumably to initiate the differentiation process leading to free antibody production. A teleological evolutionary question has recently been raised. Why is it "necessary" to have interacting "T" and "B" cell systems which were probably evolved separately (as their thymic and bursal equivalent ontological origins suggest). It is likely that the main result of interaction is to produce a quantitatively greater immunological response and perhaps by antigenic concentration to circumvent tolerogenesis.

An important question which is touched on in several papers but which remains to be answered is whether immunological specificity resides primarily in the "T" or "B" cell or in both. Jerne has suggested elsewhere that transplantation antigens may act by a process analogous to accelerated Darwinian evolution in selecting lymphocytes with somatic mutations favourable to the host's immunological defences and eliminating dangerous auto-reactivity. The large repertoire of different antibodies which the individual apparently makes in response to a single antigen (assuming the antigen itself is not equally complex) certainly suggests a series of separate somatic mutations. If Jerne's attractive hypothesis proves to be substantially correct then it is necessary to postulate that the high rate of lymphocyte death and the selectional role of transplantation antigens characteristic of the thymus and essential for the hypothesis are also characteristic of the bone marrow. If not, immunological specificity is the prerogative of "T" cells alone.

It is regrettable but understandable that much of the discussion in the proceedings had to be omitted. Bitter experience has shown that it is very difficult to obtain the consent of all contributors to a single version and at the same time not to exceed the publisher's deadline. Following meetings, authors unfortunately continue to gather wisdom so that when their fossilized *ad lib.* performance is presented to them many changes are demanded. There is, however, a copious author index and a useful subject index. This book represents a valuable review by many of the leaders in the field of immunological cell differentiation.

R. HARRIS

CORRESPONDENCE

Biological Data

SIR,—In its 1969 *International Compendium of Numerical Data Projects*, the Committee on Data for Science and Technology of the International Council of Scientific Unions listed three handbooks under the designation "Biology" as constituting the total data-collecting activities of life scientists. It is impossible to imagine that this reflects a true picture of the number of projects throughout the world that systematically extract, evaluate, and publish data in the biological sciences.

As a first step toward determining current data-collecting activities, the International Union of Biological Sciences, through its representative to CODATA, is attempting to survey existing data programs, compilations, and centres. The IUBS objectives in

obtaining this information are threefold: (1) The immediate benefit will be to reveal the kinds of biological data being collected and where they can be obtained. (2) At a later date, information gaps can be identified and steps taken to provide the required data. (3) Eventually, the coordination of data-collection efforts can be considered for the purpose of eliminating costly duplication.

If you are engaged in a data-collecting activity in any subfield of biology, your cooperation in contacting me as soon as possible would be greatly appreciated.

Yours faithfully,

PHILIP L. ALTMAN

*IUBS Representative to CODATA,
9650 Rockville Pike,
Bethesda, Maryland 20014*

Announcements

University News

Professor H. Thorpe has been appointed to the chair and headship of the Department of Geography, **University of Birmingham**.

Dr M. J. Frazer, Polytechnic of North London, has been appointed to the chair of chemical education in the School of Chemical Sciences, **University of East Anglia**, and **Dr N. Riley** has been appointed to a personal chair in the School of Mathematics and Physics.

Mr I. G. Macdonald has been appointed Fielden professor of pure mathematics in the **University of Manchester**, in succession to **Professor J. F. Adams**.

Appointments

Dr E. T. Goodwin, superintendent of the Division of Numerical Analysis and Computing, has been appointed a deputy director at the **National Physical Laboratory**.

International Meetings

January 27–28, **Ship Technology in the 1980s**, London (The Executive Secretary, The Royal Society, 6 Carlton House Terrace, London SW1Y 5AG).

February 3, **Microbiological Aspects of the Breakdown of Chemicals in the Environment**, London (Assistant Secretary, SCI, 14 Belgrave Square, London SW1X 8PS).

February 3–5, **Energy, Man and the Environment**, Zurich (John G. Cartwright, Gottlieb Duttweiler Institute for Economic and Social Studies, Park "Im Gruene", Ruschlikon near Zurich, Switzerland).

February 8, **The Determination of Fluorine**, Sheffield (Society for Analytical Chemistry, 9/10 Savile Row, London W1X 1AF).

February 22–23, **Kinetic Data from Thermal Analysis**, Ormskirk (Society for Analytical Chemistry, 9/10 Savile Row, London W1X 1AF).

February 29–March 2, **Electro-Optical Systems Design** (Dr L. R. Baker, Optical Systems Division, Sira Institute, Chislehurst, Kent BR7 5EH).

March 2–3, **Computer Chromatography and Associated Techniques**, Mainz (Miss Sarah R. Burleton, Executive Secretary, GC Discussion Group, Institute of Petroleum, 61 New Cavendish Street, London W1M 8AR).

March 6–10, **Analytical Chemistry and Applied Spectroscopy**, Cleveland (Oswald E. Wilkinson, 1972 Pittsburgh Conference, Alcoa Technical Center, PO Box 2970, Pittsburgh, Pennsylvania 15230, USA).

March 7–9, **Core-Mantle Interface**, Melbourne, Florida (American Geophysical Union, 2100 Pennsylvania Avenue NW, Washington DC 20037, USA).

March 8–9, **Climatic Resources and Economic Activity**, Aberystwyth (Dr J. A. Taylor, Department of Geography, Llandinam Building, Penglais, Aberystwyth, Cardiganshire).

March 8–10, **Molecular Studies in Viral**

Neoplasia, Houston (Mrs Jane Brandenberger, Information Coordinator's Office, University of Texas M.D. Anderson Hospital, Texas Medical Center, Houston, Texas 77025, USA).

March 9, **New Aspects of Mechanism and Synthesis in Organic Systems**, London (Dr John F. Gibson, The Chemical Society, Burlington House, London W1V 0BN).

March 13–15, **Instrumentation in Astronomy**, Tucson (Technical Program Committee, SPIE National Offices, PO Box 288, Redondo Beach, California 90277, USA).

March 13–17, **California Membrane Conference**, Squaw Valley, California (C. Fred Fox, California Membrane Conference, c/o Department of Bacteriology, University of California, Los Angeles, California 90024, USA).

March 14, **Acoustic Emission**, London (Meetings Officer, Institute of Physics, 47 Belgrave Square, London SW1).

March 15, **Filamentary Conduction**, London (Meetings Officer, Institute of Physics, 47 Belgrave Square, London SW1X 8QX).

March 19–22, **Rationalization of the Processing Chain—Fibres to Textiles**, Brussels (Dr P. A. Arias-Soto, EVAF Textile Division, 22 Square Robert Goldschmidt, 1050 Brussels, Belgium).

March 20–23, **American Society for Neurochemistry Meeting**, Seattle, Washington (Dr W. L. Stahl, Division of Neurology, University of Washington School of Medicine, Seattle, Washington 98105, USA).

March 21–23, **International Medium Voltage Earthing Practices**, London (Geraldine Walker, Institution of Electrical Engineers, Savoy Place, London WC2R 0BL).

March 21–24, **Oceanology International 72**, Brighton (Robert Clegg, BPS Exhibitions Ltd, 4 Seaford Court, 220–222 Great Portland Street, London W1N 5HH).

March 22–24, **Non-Linear Dielectrics including Ferroelectrics**, Cambridge (Dr C. W. Smith, Department of Electrical Engineering, University of Salford, Salford M5 4WT, Lancashire).

March 22–24, **Accuracy in Spectrophotometry and Luminescence Measurements**, Gaithersburg, Maryland (James I. Shultz, B228 Chemistry Building, National Bureau of Standards, Washington DC 20234, USA).

March 27–28, **The Health Challenge in Foods**, Reading (The Secretary, National College of Food Technology, St George's Avenue, Weybridge, Surrey).

March 27–29, **Ferroelectrics and their Applications**, Edinburgh (Meetings Officer, Institute of Physics, 47 Belgrave Square, London SW1X 8QX).

March 28–30, **Metalclad Switchgear**, London (Manager, IEE Conference Department, Savoy Place, London WC2R 0BL).

Reports and Publications

not included in the *Monthly Books Supplement*

Great Britain and Ireland

Microbiological Research Establishment, Porton. Abstracts of Work published in 1970. Pp. 22. (Porton, Nr. Salisbury: Microbiological Research Establishment, 1971.) [1311]

The Natural Environment Research Council. Publications Series B, No. 2: Tree, Forest and Woodland Research. Pp. 20. (London: The Natural Environment Research Council, 1971.) [1411]

The National Central Library. 55th Annual Report of the Executive Committee for the year ending 31 March 1971. Pp. 27. (London: The National Central Library, 1971.) [1511]

Our Proper Concern. By Charles Curran. (A Speech given at a Meeting of the Radio and Television News Directors' Association, Boston, Mass., 1 October 1971.) Pp. 12. (London: BBC, 1971.) [1511]

Department of the Environment. Archaeological Excavation, 1970. Pp. 93+4 plates. (London: HMSO, 1971.) 50p net. [1511]

Natural Environment Research Council. Institute of Geological Sciences. British Regional Geology. The Welsh Borderland. By J. R. Eap and B. A. Hains. Third edition. Pp. xi+118+11 plates. (London: HMSO, 1971.) 40p net. [1511]

Proceedings of the Royal Irish Academy, Vol. 71, Section A, No. 6: Combustible Vortex Rings. By P. D. McCormack. Pp. 73-84+4 plates. 36p. Vol. 71, Section B, No. 14: Meibothic Harpacticoida on the East Coast of Ireland. By C. E. O'Riordan. Pp. 191-210. 25p. (Dublin: Royal Irish Academy, 1971.) [1511]

University Grants Committee. First Employment of University Graduates, 1969-70. Pp. 51. (London: HMSO, 1971.) 68p net. [1511]

Bulletin of the British Museum (Natural History). Entomology, Vol. 26, No. 6: The Elmidae (Coleoptera) of Trinidad and Tobago. By H. E. Hinton. Pp. 245-265+9 plates. (London: British Museum (Natural History), 1971.) £1.60. [1611]

Proceedings of the First International Symposium on Biological Control of Weeds, March 1969. Edited by Dr. F. J. Simmonds. (Miscellaneous Publication No. 1.) Pp. vii+110. (Farnham Royal, Slough: Commonwealth Agricultural Bureaux, 1970.) £2. [1811]

The Natural Rubber Producers' Research Association. Rubber Developments Supplement, 1971. No. 7: Natural Rubber in Asphalt Pavements. By L. Mullins. Pp. 18. (Welwyn Garden City: The Natural Rubber Producers' Research Association, 1971.) [1811]

Natural Environment Research Council. Institute of Coastal Oceanography and Tides, 1970/1971. Pp. 34. (Birkenhead: Institute of Coastal Oceanography and Tides, 1971.) [1911]

The Control of Human Aging: a Synopsis. By Richard Bailey. Pp. 32. (London: The Reabilities Trust, 1971.) [1911]

Co-operative College Papers, No. 14: Robert Owen and his Relevance to our Times. (Addresses contributed during the Robert Owen Bi-centenary Summer School, July 17th to 23rd, 1971.) Pp. 63. (Loughborough: Education Department, Co-operative Union, Ltd., 1971.) [1911]

National Institute for Research in Dairying (University of Reading)—Report 1969-70. Pp. 180. (Shinfield, Reading: National Institute for Research in Dairying, 1971.) £1.20. [1911]

Water Resources Board. The Measurement of River Water Temperature. By R. W. Herschy. (TN5 revised.) Pp. 18. The Magnitude of Errors at Flow Measurement Stations. By R. W. Herschy. (TN11 revised.) Pp. 30. (Reading: Water Resources Board, 1971.) [1911]

Queen Elizabeth College, University of London. Department of Physics Research Report, 1969-1971. Pp. 38. (London: Queen Elizabeth College, 1971.) [1911]

The Institution of Gas Engineers. Communications. No. 858: Second Report of the Education and Training Committee, 1970-71. Pp. 15. 50p. No. 859: Gas for Radiant Space Heating. By F. M. H. Taylor. Pp. 11. 50p. No. 860: A Study of Unvalved Pulse Combustors. By F. E. J. Briffa, P. W. Staddon, R. N. Phillips, and D. R. Romaine. Pp. 12. 50p. No. 861: Fundamentals of Methane Combustion. By G. Dixon-Lewis, et al. Pp. 9. No. 862: Institution Gas Research Fellowship Report, 1967-70. Pp. 11. 50p. No. 863: Recent Developments in Flueing. By J. Emerson and M. J. Randall. Pp. 17. £1. No. 864: Marketing Research in the Natural Gas Industry. By F. J. Johnson. Pp. 14. 50p. (London: The Institution of Gas Engineers, 1971.) [2211]

The Gas Council. Research Communications. No. 180: Report on the Gas Council Research Scholarships, 1971. Pp. 8. 50p. No. 181: Recent Developments in Domestic Heating by Hot Water. By B. S. Cheyney and E. A. K. Patrick. Pp. 16. 50p. No. 182: A Study of the Performance of Automatic Packaged Gas Burners. By M. L. Hoggarth, D. A. Jones and K. F. Pomfret. Pp. 15. No. 183: 62nd Report of the Joint Refractories Committee, 1970-71. Pp. 24. £1. No. 184: The Prediction of Heat Transfer and Thermal Performance in Gas-Fired Processes. By R. M. Davies, D. M. Lucas and Mrs. H. E. Toth. Pp. 19. £1. No. 185: Some Characteristics of Practical Spark-Ignition Systems. By B. Ekins and J. R. Wilson. Pp. 14. No. 186: Soot in Laminar Gas Flames. By Stuart B. Reed and Frank G. Roper. Pp. 17. No. 187: The Properties of British Natural Gas. By A. B. Densham and R. M. Gibbons. Pp. 13. No. 188: Inspecting Inspectors. By R. Chrisp, R. F. Lumb, B. Phelps and R. B. Gibbon. Pp. 14. 50p. No. 189: A Review of Computer Programs for Network Analysis (Developed at London Research Station). By A. E. Fincham. Pp. 11. 50p. No. 190: Safety Shut-Off Systems for Gas Burners. By S. H. Hutt, D. J. Moppett and K. Stein. Pp. 13. 50p. No. 191: Terminal Design for Fan-Assisted Balanced Flues. By N. C. Ross and J. C. Tipping. Pp. 15. 50p. (London: The Gas Council, 1971.) [2211]

Engineering Industry Training Board. Booklet No. 14: The Training of Technicians. Pp. 52. (Watford: Engineering Industry Training Board, 1971.) [2311]

Philosophical Transactions of the Royal Society of London. B: Biological Sciences, Vol. 263, No. 848 (18 November 1971): Fine Structure and Development of the Zygospore of *Rhizopus sexualis* (Smith) Callen. By Lilian E. Hawker and A. Beckett. Pp. 71-100+plates 5-15. (London: The Royal Society, 1971.) £1.75; \$4.55. [2311]

University College of Wales, Aberystwyth. Report of the Welsh Plant Breeding Station for 1970. Pp. 121. (Plas Gogerddan, Near Aberystwyth: Welsh Plant Breeding Station, 1971.) 50p. [2411]

World Meteorological Organization. Manual for Depth-Area-Duration Analysis of Storm Precipitation. Pp. xv+114. Technical Notes. No. 97: Practical Soil Moisture Problems in Agriculture. Pp. xv+69. No. 101: Meteorology and Grain Storage. By C. V. Smith. Pp. xvi+65. No. 105: Artificial Modification of Clouds and Precipitation. By M. Neiburger. Pp. 33. (Geneva: World Meteorological Organization, 1968 and 1969.) [2910]

Seismic Methods for Monitoring Underground Explosions—1971. Progress Report. By Dr. David Davies. Pp. 24. (Stockholm: Stockholm International Peace Research Institute, 1971.) [2910]

Australian Academy of Science. Year Book, June 1971. Pp. 124. (Canberra: Australian Academy of Science, 1971.) [811]

Unesco. Technical Papers in Hydrology, No. 8:

Flood Studies—an International Guide for Collection and Processing of Data. Edited by F. Snyder, A. Sokolov and K. Szesztay. Pp. 49. (Paris: Unesco; London: HMSO, 1971.) 16 francs; £1.20; \$4. [811]

Australia: Commonwealth Scientific and Industrial Research Organization. Annual Report of the Division of Tropical Pastures, 1970-71. Pp. 104. (Brisbane: CSIRO, 1971.) [1011]

CSIRO, Australia, Division of Soils. Technical Papers. No. 4: Fortran Programs for Use in Fluorescent X-ray Analyses of Silicates, Soils and Vegetable Matter. By A. C. Oertel. Pp. 24. No. 5: Computer Programs for Use in X-ray Spectrography—Determination of Trace Elements and of Dead-Time of Counters. By A. C. Oertel. Pp. 16. (Melbourne: CSIRO, 1971.) [1011]

Queen Victoria Museum and Art Gallery, Launceston, Tasmania. Annual Report 1969/1970. Pp. 8+2 plates. Records of the Queen Victoria Museum. No. 37: On the Occurrence in Tasmania and on Flinders Island of *Brachygalaxias Eigenmanni*, 1928 (Pisces: Galaxiidae), with Descriptions of Two New Species. Pp. 14. No. 38: Sea Turtles Round Tasmania. By R. H. Green. Pp. 4. No. 40: The Birds of King Island. By R. H. Green and A. M. McGarvie. Pp. 42. No. 41: Parasites of Tasmanian Native and Feral Fauna. Part 1: Arthropoda. By R. H. Green and B. L. Munday. Pp. 16. (Launceston: Queen Victoria Museum, 1971.) [1011]

Smithsonian Contributions to Zoology, No. 100: Taxonomic Study of the Known Pupae of the Genus *Anthrax* (Diptera: Bombyliidae) in North and South America. By Norman Marston. Pp. 18+4 plates. (Washington, DC: Smithsonian Institution Press, 1971. For sale by US Government Printing Office.) \$0.35. [1111]

Background Study for the Science Council of Canada. Special Study No. 13: Earth Sciences Serving the Nation. Pp. 363. (Ottawa: Information Canada, 1971.) \$4.50. [1111]

US Department of the Interior: Geological Survey. Water-Supply Paper 1866-A: Floods of December 1964 and January 1965 in the Far Western States. Part 1: Description. By A. O. Waananen, D. D. Harris and R. C. Williams. Pp. x+265. (Washington, DC: Government Printing Office, 1971.) \$1.25. [1111]

Bulletin of the American Museum of Natural History, Vol. 145, Article 3: Biologies of African Alodapine Bees (Hymenoptera, Xylocopinae). By Charles D. Michener. Pp. 219-302. (New York: American Museum of Natural History, 1971.) \$3.15. [1111]

The Israel Academy of Sciences and Humanities. Proceedings—Section of Sciences, No. 18: Quasars and Pulsars. By Y. Ne'eman. Pp. 18. (Jerusalem: The Israel Academy of Sciences and Humanities, 1971.) IL2.50; \$1.25. [1111]

Canada: Department of Energy, Mines and Resources. Geological Survey of Canada. Bulletin 192: Contributions to Canadian Paleontology. By A. E. H. Pedder, A. C. Lenz, D. E. Jackson, A. R. Ormiston, W. W. Nassichuk and T. P. Chamney. Pp. 108 (18 plates). \$6. Paper 70-33: Metamorphic Map of the Canadian Cordillera. By J. W. H. Monger and W. W. Hutchison. Pp. v+59. \$2. Paper 71-4: Abstracts of Publications in Scientific Journals by Officers of the Geological Survey of Canada, April 1970 to March 1971. Pp. 51. \$1.50. (Ottawa: Information Canada, 1971.) [1211]

Science Policy Research and Teaching Units/Unités de Recherche et d'Enseignement en Politique Scientifique—Europe and North America/Europe et Amérique du Nord, 1967-1970. Pp. 378. (Paris: Unesco; London: HMSO, 1971.) 20 francs; £1.50. [1211]

New Students and New Places: Policies for the Future Growth and Development of American Higher Education. (A Report and Recommendations by The Carnegie Commission on Higher Education, October 1971.) Pp. 158. (Hightstown, NJ: McGraw-Hill Book Company, 1971.) \$3.50. [1311]

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